HYDROXAMIC ACIDS DERIVED FROM N-HYDROXY-α-AMINO ACIDS: SYNTHESES AND APPLICATIONS

H.J.M. ZEEGERS

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CHAPTER

CHAPTER I

INTRODUCTION

INTRODUCTION

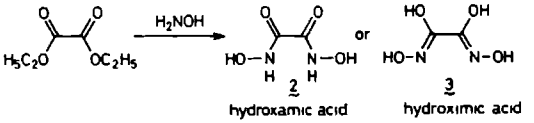
1.1 GENERAL

Hydroxamic acids (1) are compounds characterized by one or more oxidized amide bonds, *i.e.* the hydroxamic acid functions.[1-5]

R^1, R^2 = H, alkyl, aryl

1

The first synthesis of a hydroxamic acid was described in 1869 by H. Lossen.[6] He reported that the reaction between diethyloxalate and hydroxylamine yielded an acidic compound which he named oxalohydroxamic acid :



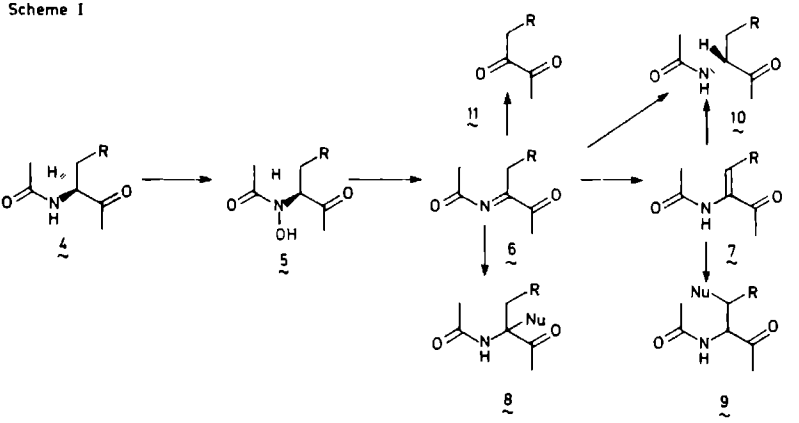
At that time it was difficult to distinguish between the two possible structures 2 and 3. During the past four decades the development and application of spectroscopic methods made it possible to establish the structure of the hydroxamic acid firmly as 2.

Since the isolation in 1944 of aspergillic acid[3,7] (see 2.3), the first example of a naturally occurring hydroxamic acid, a substantial number of natural products which contain one or more hydroxamic acid functions have been found, mainly from microbial sources.[2,3] All these compounds belong invariably to the class of the so-called secondary metabolites. In general, secondary

metabolites are distinct from primary metabolites in that they are frequently of relatively complex structure; in addition their distribution is restricted and more characteristic of specific sources.[8,9] Whereas there are no sharp lines delimiting either class of compounds, primary metabolites are nearly universal in their distribution; they are the products of, and participants in, the cellular activities of nearly all living organisms, from microorganisms to man.

Several of the naturally occurring hydroxamic acids are derivatives of N-hydroxy-α-amino acids.[2,3,4b,10-13] The synthesis and possible applications of hydroxamic acids derived from N-hydroxy-α-amino acids, *e.g.* 5, will be the subject of this thesis. It has been postulated[14] that N-hydroxy-α-amino acids 13 are intermediates in a pathway linking L-α-amino acids and several "uncommon" amino acids. It seems reasonable that a similar biogenetical and/or chemical relationship holds for acylated α-amino acids and compounds containing uncommon amino acid residues, hydroxamic acids 5 acting as crucial intermediates (scheme I).

Scheme I

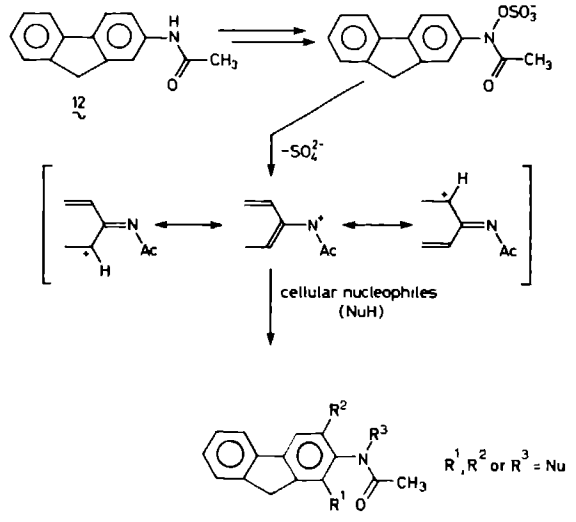


This hypothesis is based on the following considerations.

First, as has been demonstrated before[14], only the route depicted in scheme I links N-hydroxy-α-amino acid derivatives 5 with the other uncommon amino acid derivatives 6-10 (see for comparison ref. 15-17).

Second, it has been shown that N-hydroxylation of amino acids and amides (4 → 5) is a familiar degradative pathway among the metabolic processes of many organisms.[18-27] The reaction is not limited to plants and microorganisms as N-hydroxy peptides have been found likewise in human and animal tumors.[28,29] The most notorious example of N-hydroxylation concerns the activation of 2-acetylaminofluorene 12 (scheme II), a carcinogenic aromatic amide[19,24,30]; after sulfate conjugation[30] the N-hydroxy-2-acetylaminofluorene is a potent arylating agent.

Scheme II

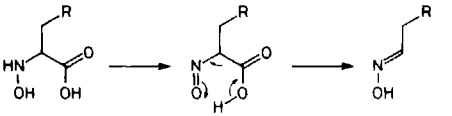


Thirdly, several of the organisms that produce fungal metabolites featuring the uncommon amino acid residues 7-10 also produce

N-hydroxyamino acid-containing metabolites.[2,3,31] Species belonging to the genus Penicillium[10,32,33], Aspergillus[12,13,34] or Streptomyces[11,35,36] are examples of such organisms.

The demonstration of N-hydroxy-α-amino acids 13 as intermediates in metabolic pathways, which has not received much attention until recently, has been impeded by the instability of these compounds, by the lack of a general synthesis and proper analytical techniques, and by their occurrence in only minute quantities in biological material. The lability of these compounds is caused by the strongly reducing properties of the free hydroxylamine. Oxidation of this functionality gives the corresponding α-nitroso compound 14 as a primary product, which decarboxylates readily to aldoxime 15.[20] (see scheme III).

Scheme III



On the other hand, N-alkylated- and N-acylated derivatives (hydroxamic acids, e.g. 5) of N-hydroxy-α-amino acids are fairly stable and have been identified as building stones of natural products.[2,3,37]

1.2 *INTRODUCTION TO THE CHAPTERS*

In this thesis several aspects of the chemistry of hydroxamic acids derived from N-hydroxy-α-amino acids 13 come up for discussion. Some general information concerning the properties and biosynthesis of hydroxamic acids is given in the second chapter. Also some structures of representative examples of naturally occurring hydrox-

amic acids are presented. The possible application of simple chiral hydroxamic acids as ligands in asymmetric, metal-catalyzed reactions is discussed in chapter III. Chapter IV reviews the crucial advances in our understanding of penicillin biosynthesis during the past decade. Further this chapter sets out our efforts to verify the postulate that an N-hydroxy peptide might be involved as an intermediate in penicillin biosynthesis. Finally, the results of experiments involving a mild conversion of α-oximino acid derivatives into corresponding primary and secondary N-hydroxy-α-amino acid derivatives are evaluated in Chapter V.

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CHAPTER II

THE CHARACTERISTICS OF HYDROXAMIC ACIDS

THE CHARACTERISTICS OF HYDROXAMIC ACIDS

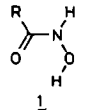
2.1 *INTRODUCTION*

The intention of this chapter is to furnish some insight into the chemistry and biological properties of hydroxamic acids in general. Before going to naturally occurring hydroxamic acids some attention is payed to the chemistry of hydroxamic acids. This knowledge is indispensable for understanding their biological mode of action and for recognizing their potential applicability in organic synthesis.

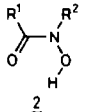
2.2 *CHEMICAL PROPERTIES OF HYDROXAMIC ACIDS*

2.2.1 *Acidity*

As mentioned in the first chapter the oxidized amide bond is the outstanding chemical feature of hydroxamic acids. This functional group is a weak proton donor.[1] Whereas there is some ambiguity in the current literature, whether primary hydroxamic acids 1 act as NH- or OH-acids,[1a,2] substituted (secondary) hydroxamic acids 2 are obvious OH-acids.



1
primary
hydroxamic acid



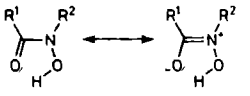
2
secondary
hydroxamic acid

R, R^1, R^2 = alkyl, aryl

The pKa-values of primary hydroxamic acids 1 vary[1,3,4] between 7 and 10; secondary hydroxamic acids exhibit[3,5] a pKa-value of

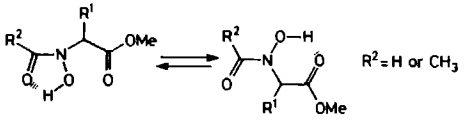
approximately 11. Unfortunately, there are some discrepancies in the reported pKa-values, presumably brought about by differences in the experimental techniques used to determine the values.[1,3-5]

Scheme I



The planarity of the hydroxamic acid group and its bond lengths indicate partial N-C(O) double bond character as found in amides[6] (see scheme I). The existence of stable rotamers, as a result of hindered rotation about the N-C(O) bond, has been demonstrated.[6,7] Usually the hydroxamic acids exist in a planar *cis(Z)*-configuration, which is stabilized by an intramolecular hydrogen bond.[6,7] (scheme I). However, there are some exceptions.[6,7] Kolasa[7] investigated the conformational behaviour of some N-formyl- and N-acetyl-N-hydroxy-α-amino acid esters by ^{1}H-NMR and IR-spectroscopy. These compounds exist as a mixture of *Z/E* rotamers (see scheme II), the ratio of which depends on the solvent used and on the nature of the substituents R^1 and R^2.

Scheme II



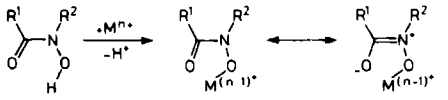
cis(Z) *trans(E)*

The free enthalpy of activation (ΔG*) for this interconversion has been estimated at about 16 kcal mol^{-1}, which is ca. 5 kcal mol^{-1} lower than ΔG* of the corresponding interconversion in amides.[7,8]

2.2.2 *Formation of metal complexes*

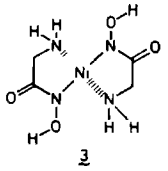
Hydroxamic acids form isolable alkali metal and silver salts[9-11,45] and also stable complexes with a large number of transition metals.[3,4,12-19] The ability of hydroxamic acids to dissociate allows for attachment of a metal ion to the bidentate anion, creating a stable five-membered ring (see scheme III).

Scheme III



Spectroscopic methods have shown that both oxygen atoms of the donor ligand are involved in chelation[17]; delocalization over the chelate ring results in significant double bond character of the C-N bond.[16,18] A few years ago, however, the first case of coordination of the NOH^- group via the nitrogen atom was reported[19] (see scheme IV). It concerns a square-planar nickel complex 3 with *trans* geometry.

Scheme IV



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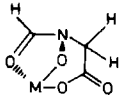

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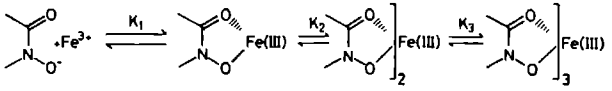
If a molecule, besides a hydroxamic acid function, also contains a carboxyl group it can act as a terdentate ligand as was demonstrated for N-formyl-N-hydroxyglycine 4 (see scheme IV).[16]

Effective competition between protons and chelating cations makes complex-formation a pH-dependent process.[12a,15,19b] Binding occurs in a stepwise manner as the pH is raised. Complexes with the ferric ion are the best known; in this case (scheme V) the 1:1

structure formed at low pH is transformed into a 3:1 complex as the pH approaches neutrality.[12]

Scheme V



5

Formation (or stability) constants can be defined for the individual steps.[15,20] The formation constant for the overall reaction is numerically equal to the product of the constants for the three constituent processes ($K_S = K_1.K_2.K_3$). The intensely burgundy-coloured ferric complexes 5 have very high complexation constants (K_S up to 10^{28}); three hydroxamate groups create a complex with an octahedral configuration.[12,13] When the three hydroxamate functions are present in the same molecule, as in the case of some siderophores[12,13] (*vide infra*), the complex (K_S 10^{29} or higher) tends to retain the 3:1 structure even at low pH.

Because hydroxamic acids exhibit little cation selectivity, with the exception of a distinct preference for ferric ion, complexation with several metal ions is strong enough[15,16] to be of great utility. To illustrate this, a few examples are presented.

Copper(II) complexes have been used for identifying and purifying hydroxamic acids.[21-23] Treatment of a hydroxamic acid with alcoholic copper(II) acetate results in the precipitation of a copper(II) complex. The hydroxamic acid can be liberated from the complex by bubbling H_2S through a solution of the complex; copper sulfide precipitates and can be removed easily by filtration.[22] The ability of hydroxamic acids to form stable complexes with zinc ions underlies the method which Nishino and Powers[24] developed for the purification of zinc metalloproteases by column chromatography. To

that aim they synthesized an affinity adsorbent containing a hydroxamic acid moiety. The adsorbent appeared to be specific for several zinc metalloproteases, and a variety of conditions was developed for elution of the enzymes from the column.[24]

During the isolation of N-hydroxy peptides from homogenized tumour-tissue advantage was taken of the relatively high affinity[15] of hydroxamic acids for aluminum(III).[25,26] Under alkaline conditions the peptide fractions containing a hydroxamate moiety were completely retained on an alumina column, whereas they could be eluted from the column using an acidic eluent.

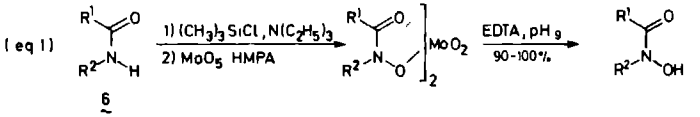
In contrast with the ferric ion ($K_1 \approx 10^{11}$, see scheme V), the ferrous ion is bound only loosely to hydroxamic acids[15] ($K \approx 10^5$). This special feature can be used to explain the biological function of many hydroxamic acids. In section 2.3 more attention will be payed to this topic.

2.2.3 *Syntheses and reactions*

The most convenient methods for the synthesis of hydroxamic acids will be discussed in the chapters III, IV and V. In this section just a brief outline of some oxidative methods for their synthesis is given.

Whereas N-acylation of hydroxylamines has long been used for the synthesis of hydroxamic acids, the direct oxidation of amides has only recently come under scrutiny. Following the discovery that trimethylsilylation of secondary amides activates them towards oxidation to hydroxamic acids by the peroxo-molybdenum complex MoO_5.HMPA,[27] the scope of this reaction (eq.1) has been investigated in more detail by Sammes.[28] Although he has used rather simple amides, the yields of the oxidation step are generally moderate to

low (≤ 48%). Liberation of the uncomplexed hydroxamic acids was

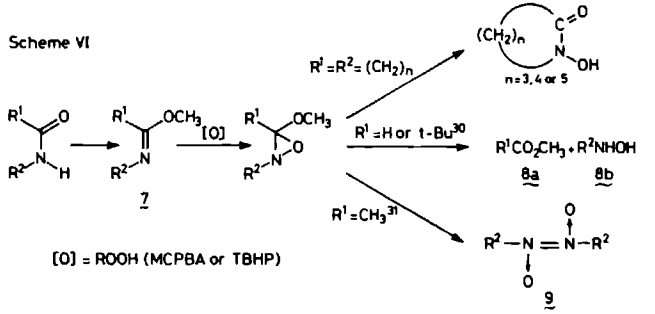


6

accomplished by extraction of the molybdenum ion with warm ethylene diaminetetraacetic acid (EDTA) at pH 9. Treatment of the molybdenum complexes with hydrogen sulphide, aimed at the precipitation of the metal as its sulphide, causes the undesirable reduction of the hydroxamic acid to the parent amide 6.

Some attempts have been described[29-31] to convert imino ethers 7 into hydroxamic acids (scheme VI).

Scheme VI

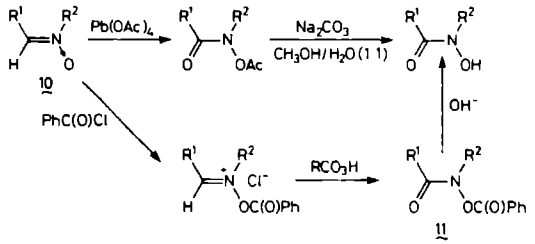


Oxidation of an acyclic imino ether followed by hydrolysis of the resulting alkoxyoxazirane leads to the corresponding ester 8a and hydroxylamine 8b[30] or to the nitroso dimer 9.[31] The product formation seems to be dependent on the nature of the residue R^1.[31] In this way only cyclic hydroxamic acids can be synthesized in low yields.[29,30]

A more elegant method for the preparation of hydroxamic acids is the oxidation of aldonitrones[32] 10 or derivatives thereof[33] (scheme VII). Acylation of the nitrone facilitates its oxidation; the formation of an O-acyl hydroxamic acid 11 offers the advantage

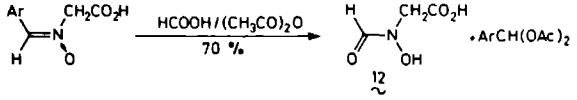
that this compound, in contrast with free hydroxamic acids,[34] is

Scheme VII



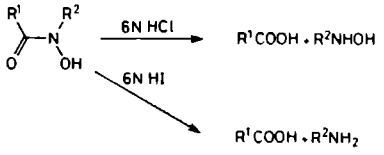
protected from further oxidation by the peracid. Searching for a more direct conversion of a nitrone into N-formyl-N-hydroxyglycine 12, Schoenewaldt *et.al.* found that nitrones can be cleaved under concomitant formylation by treatment with formic acetic anhydride at room temperature (eq.2).[35]

(eq 2)



The remaining part of this section is completely dedicated to the chemical reactivity of hydroxamic acids. Due to the presence of a hydroxyl group on the nitrogen atom these compounds behave quite different from amides in many aspects. An exception is the hydrolysis of the hydroxamic acid bond, which can be accomplished under both

Scheme VIII

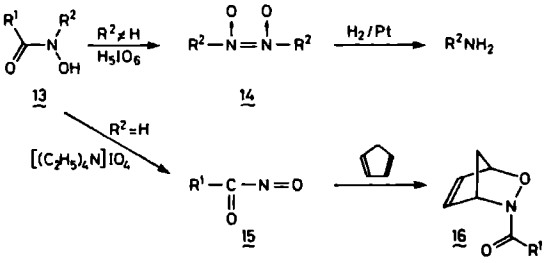


basic and acidic conditions.[2] Acidic conditions (6N HCl, scheme VIII) are preferable in those cases where elimination reactions (*vide infra*) can interfere. Hydrolysis with 6N HI converts the

hydroxylamine group into a primary amino group.[12,13]

The hydroxamic acid bond is susceptible to oxidation and reduction. These reactions have mainly been used for the characterization of naturally occurring hydroxamic acids.[12,13] Periodic acid is the reagent of choice for oxidation of the hydroxamic acid bond since the amide bond and other even more sensitive bonds (*e.g.* the ester linkage) remain unaffected (scheme IX).

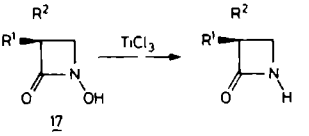
Scheme IX



The products of the "hydrolysis by oxidation" of secondary hydroxamic acids are an acid derived from the acyl moiety and a dimeric nitroso compound 14.[12,13] C-Nitroso carbonyl compounds 15 are formed as transient intermediates in the oxidation of primary hydroxamic acids (13, R^2=H) with tetraethylammonium periodate.[12,36,37] These dienophiles can be trapped by cyclopentadiene to give the corresponding cycloadducts 16.[36,37]

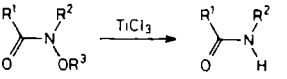
Treatment with zinc powder in acetic acid is the most frequently used method for the reduction of hydroxamic acids to the corresponding amides.[12,13,21,23] Another reagent which is very efficient for this purpose is the combination of hydrogen gas and Raney nickel.[12,38] A mild method for the reduction of the N-O bond of, among others, substituted N-hydroxy-2-azetidinones 17 (eq.3) was developed by Miller.[38]

(eq 3)



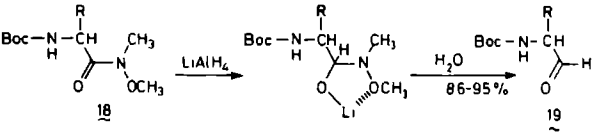
17

(eq 4)



A prerequisite is the presence of an acidic chelation site in the molecule, *i.e.* R^2 and/or R^3 (eq.4) must be a proton.[38] Under strongly reducing circumstances amino acid aldehydes 19 can be obtained from the corresponding O-alkylated primary hydroxamic acids 18 (eq.5).[39]

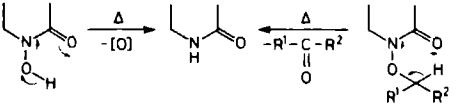
(eq 5)



18 19

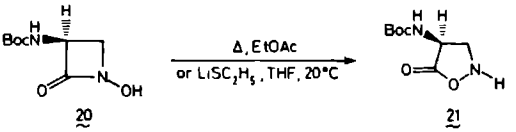
A characteristic reaction of hydroxamic acids, in particular cyclic ones (five- and six-membered rings), is thermal reduction to the corresponding amides. O-alkyl derivatives are particularly prone to this reaction (scheme X).[40]

Scheme X



Under analogous conditions N-hydroxy-2-azetidinone 20 isomerizes in low yield (20%) into the corresponding isooxazolidin-5-one 21 (eq. 6).[41] The same compound 21 can be obtained in 83% yield by treatment of 20 with a catalytic amount of lithium ethanethiolate at room temperature.[42]

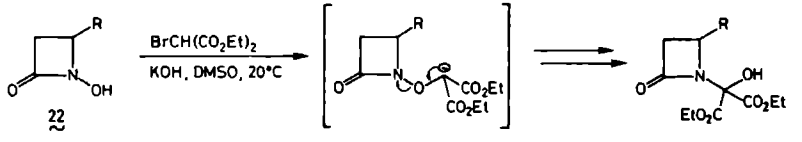
(eq 6)



20 21

Introduction of a negative charge can also induce a rearrangement of hydroxamic acid derivatives. Recently Miller described the formation of a carbinolamine 23 by a [1,2]-anionic rearrangement of substituted N-hydroxy-2-azetidinones 22 (eq.7).[43]

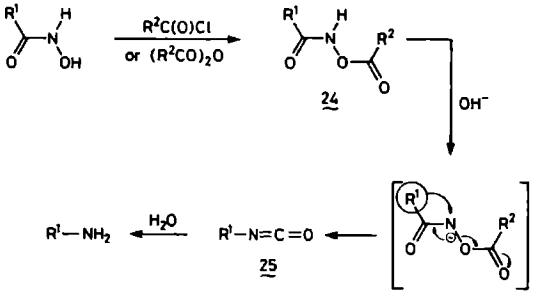
(eq 7)



22 23 35-55%

Another example is a reaction known as the Lossen rearrangement[44]; O-acyl derivatives of hydroxamic acids 24 give isocyanates 25 when treated with a base,[2] or sometimes even just on heating (eq.8).[2,45]

(eq 8)



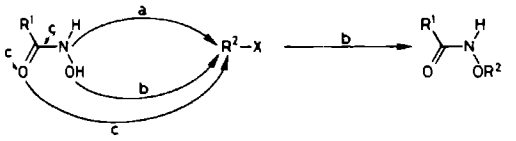
24 25

The starting compound 24 is readily accessible by acylation of the corresponding primary hydroxamic acid with an acid chloride or anhydride.[2,45] The same holds true for the synthesis of O-acylated secondary hydroxamic acids.[45,57,62]

Hydroxamic acids are sufficiently acidic to be O-methylated

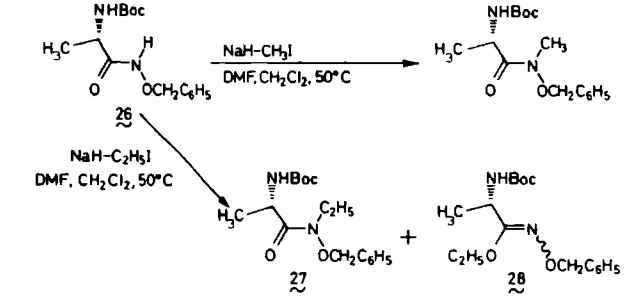
with diazomethane.[21,40,57] In most cases, however, alkyl halides are used for this conversion.[2,11,45] Unsubstituted hydroxamic acids contain three different sites where an alkylating agent can attack. Usually alkylation of the hydroxyl group predominates[2] (eq.9, route b).

(eq 9)



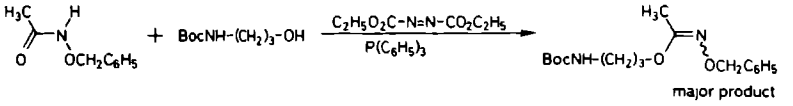
When the hydroxyl group is protected, the regioselectivity of the alkylation depends on the reagent and experimental conditions used. Some examples are presented in scheme XI, eq.10 and scheme XII.

Scheme XI



Olsen *et.al.*[46] found that clean N-alkylation of the hydroxamate function of Boc-Ala-NHOBzl 26 is apparently unique for methyl iodide as the alkylating agent. Alkylation with ethyl iodide (or benzyl bromide) furnished a mixture of the N- and O-alkylated products (27 resp. 28, ratio 4:1). Intermolecular alkylation of hydroxamates with alcohols mediated by diethyl azodicarboxylate-triphenylphosphine

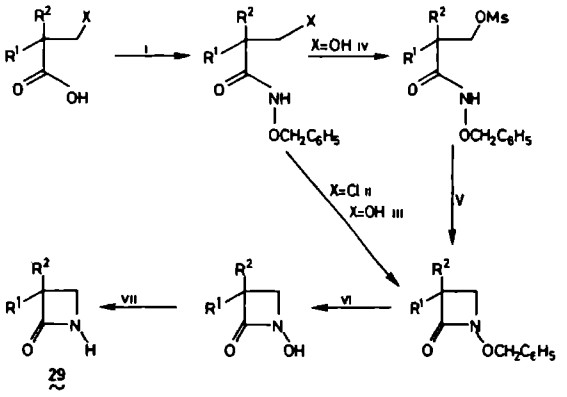
(eq 10)



major product

(Mitsunobu reagent[47]) gives mainly or entirely the O-alkylated product (eq.10).[48,49] Clean intramolecular alkylation on nitrogen has been reported in the preparation of β-lactams 29 (scheme XII).[49-56]

Scheme XII



i WSC; H_2NOCH_2Ph

ii NaH, DMF-CH_2Cl_2 (1:1), 50°C

iii EtO_2C-N=N-CO_2Et; PPh_3 or CBr_4; PPh_3; Et_3N

iv CH_3SO_2Cl; pyridine

v t-BuO^-K^+, DMF, -23°C

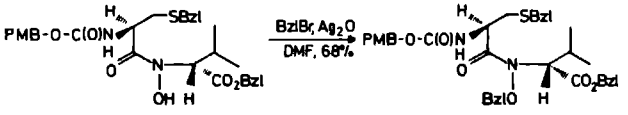
vi H_2,Pd/C

vii $TiCl_3$

WSC = water-soluble carbodiimide : [N-ethyl-N'-3-(dimethylamino)-propyl]carbodiimide

Problems concerning the regioselectivity are not encountered in the

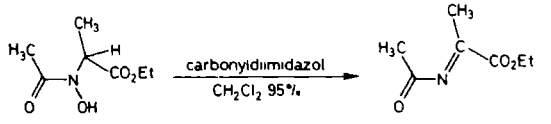
(eq 11)



alkylation of N-substituted hydroxamic acids; alkylation of the hydroxyl group is the only possibility (eq.11).[57]

The last part of this section is mainly devoted to some special chemical features of hydroxamic acids, which are derived from N-hydroxy-α-amino acids. Compounds belonging to this class, *ie* N-acyl-N-hydroxy-α-amino acid dervatives, undergo readily elimination reactions to yield the corresponding α-acylimino carboxylic acid derivatives (eq.12-14, scheme XIII). The elimination reaction can be facilitated by converting the OH-group into a better leaving group, for instance by acylation, alkylation or tosylation (eq.13 and scheme XIII).[59-66] As far as known there is only one example of the successful isolation of such an acylimine (30, eq.12)[58]:

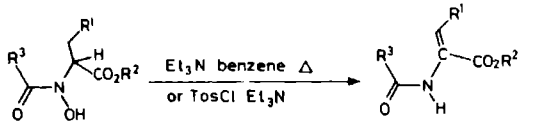
(eq 12)



30

In the absence of a nucleophile they usually tend to rearrange to the corresponding enamino acid derivative (31, eq.13 or 32, scheme XIII).[59-64]

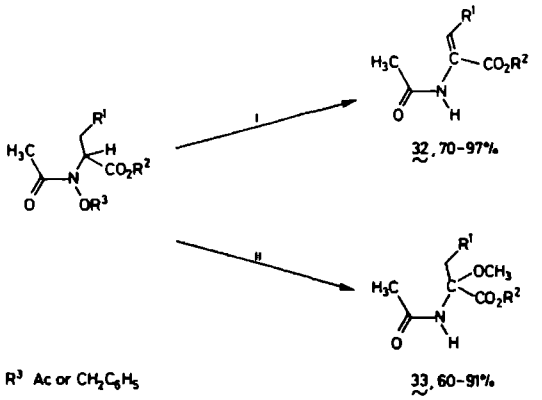
(eq 13)[60-62]



31

The acylimine can be captured in the presence of an excess of a hard nucleophile to yield the α-functionalized amino acid derivative 33 (scheme XIII).[65,66] Reaction of an acylimine with H_2O results in the formation of the corresponding α-keto carboxylic acid derivative (34, eq.14).[67]

Scheme XIII



32, 70-97%

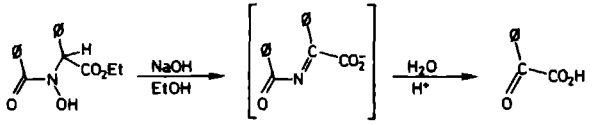
33, 60-91%

R^3 Ac or $CH_2C_6H_5$

i. DBU, CH_2Cl_2[59] or (R^3=Ac) Et_3N, benzene, Δ[62-64]

ii t-BuOK, MeOH[65,66]

(eq 14)

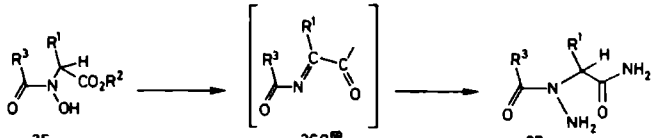


34

These methods for the synthesis of α-functionalized amino acid derivatives and α,β-dehydroamino acid derivatives are efficient and might be of general applicability.

Nucleophilic substitution at nitrogen, resulting in N-O bond dissociation, has been reported only a few times.[66,68,69] Reaction of an N-acyl-N-hydroxy-α-amino acid derivative 35 with an excess of ammonia leads to the formation of a hydrazino derivative 37 (eq.15).[68] This result can be rationalized by the intermediacy of the acylimine derivative 36; a hard nucleophile attacks the carbon

(eq 15)

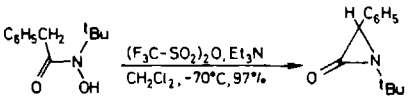


35 36 37

atom (eq.14), whereas a softer nucleophile yields the N-substituted product 37.[66,69]

A substitution reaction which certainly cannot involve the intermediacy of an acylimine derivative was described by Kirby[70] (eq.16); intramolecular attack of a carbanion on the nitrogen atom of a modified hydroxamic acid function results in cleavage of the N-O bond, under formation of an aziridinone (α-lactam) 38.

(eq 16)



38

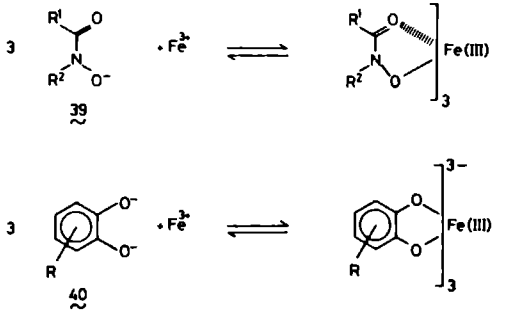
With respect to the last part of this section it may be concluded that some of the examples (eq.12-14, scheme XIII) show clearly that several routes depicted in the first scheme of chapter I are at least of chemical relevance.

2.3 *STRUCTURE AND BIOLOGICAL FUNCTION OF NATURAL HYDROXAMIC ACIDS*

The past three decades have witnessed the compilation of a catalogue of microbial iron-containing or iron-binding compounds, most of which are classified as "siderophores" (Greek for iron bearers).[12-14,71] Although the siderophores as chemical entities display considerable structural variation, most of them are either hydroxamates 39 or catecholates 40, and all exhibit a very strong affinity for Fe(III), the formation constant lying in the range of 10^{30} or higher (scheme XIV).

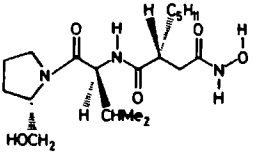
Natural hydroxamic acid containing compounds are ubiquitous (fungi, yeasts, bacteria, algae, plants) and frequently bewildering in structural variety and biological implications. Before turning

Scheme XIV



to their biological function some remarks concerning their structure will be made.

With the exception of actinonin (41)[72] they are all of the secondary type.

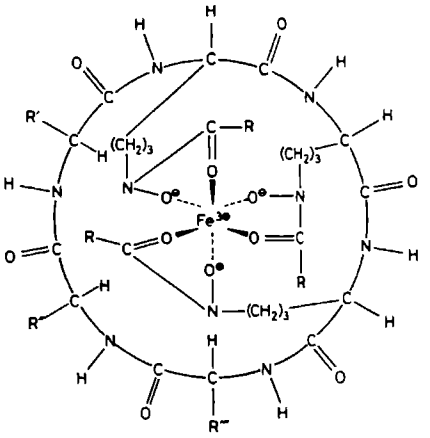


41, actinonin

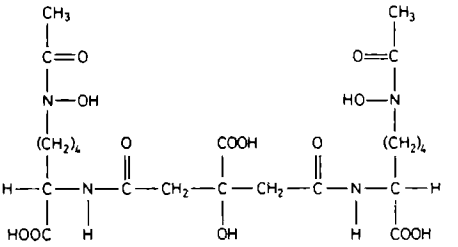
(Streptomyces roseopallidus)[72]

Most of the naturally occurring hydroxamic acids contain amino acid residues as building stones. With respect to these building blocks the compounds can be divided up into two classes encompassing either α-N-hydroxyamino acids or ω-N-hydroxyamino acids. Compounds that belong to the latter group contain, in addition to the α-amino- and carboxyl group, a terminal (ω-) hydroxylamine function. The ω-N-hydroxy derivatives of ornithine and lysine are the most commonly found N-hydroxyamino acids that occur in natural hydroxamic acids.[12-14,71,73-77] Two examples, representative for this kind of compounds, are depicted in scheme XV. The characteristic structural feature of the ferrichrome family 42 is a cyclic hexapeptide containing a tripeptide sequence of N^{δ}-acyl-N^{δ}-hydroxy-L-ornithine

Scheme XV



42, ferrichrome family (R=CH_3)
(Ustilago sphaerogena)



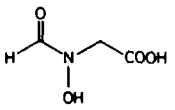
43, aerobactin
(Aerobacter aerogenes)

and a tripeptide composed of small, neutral amino acids. Aerobactin 43 is a compound that is made up of citric acid and two N^{ϵ}-acyl-N^{ϵ}-hydroxy-L-lysine residues.

As was explained before, in this thesis hydroxamic acids derived from N^{α}-hydroxy-α-amino acids are highlighted. In scheme XVI[12-14] all representatives of this class, found in nature until now, are depicted. It should be mentioned that some of them have been isolated from several sources.[12,13] N-Hydroxy peptides have also been isolated from animal and human tumours. The exact structure of these compounds has not yet been elucidated; it was estimated that approximately one out of 300-500 peptide bonds is N-hydroxylated.[25,26]

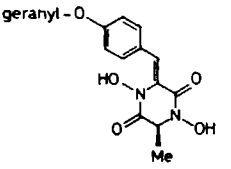
Concerning the biological function (and/or activity) of the naturally occurring hydroxamic acids it has been demonstrated that

Scheme XVI



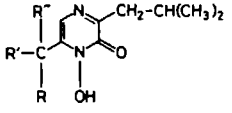
44, hadacidin

(Penicillium frequentans)[78]



45, mycelianamide

(Penicillium griseofulvum)[79]



46, aspergillic acids[13]

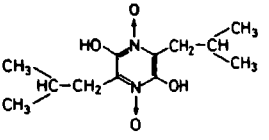
a aspergillic acid R=H, R′=C_2H_5, R″=CH_3

b neo-aspergillic acid R=R″=H, R′=$CH(CH_3)_2$

c muta-aspergillic acid R=OH, R′=R″=CH_3

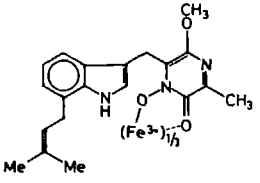
d hydroxy-aspergillic acid R=OH, R′=C_2H_5, R″=CH_3

e neo-hydroxy-aspergillic acid R=OH, R′=$CH(CH_3)_2$, R″=H



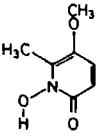
47, pulcherriminic acid

(Candida pulcherrima)[13]



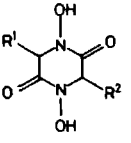
48, astechrome

(Aspergillus terreus)[80]



49, G1549

(Pseudomonas alcaligenes)[22]



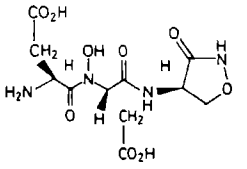
50, terramides

(Aspergillus terreus)[23]

terramide A $R^1=R^2=(R)\text{-}CH(CH_3)CH_2CH_3$

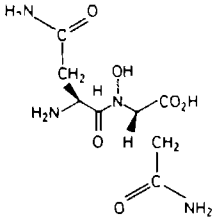
,, B $R^1=(R)\text{-}CH(CH_3)CH_2CH_3$, $R^2=CH(CH_3)_2$

,, C $R^1=R^2=CH(CH_3)_2$



51 L-α aspartyl-L-α N-hydroxy-aspartyl-D-cycloserine

(Corynebacterium kutscheri)[67]



52 L-α-asparaginyl-L-α N-hydroxy asparagine

(Mycobacterium avium)[82]

they can act variously as potent growth factors (*eg* aspergillic acid derivatives 46), antibiotics (*eg* mycelianamide 45, aspergillic acid derivatives 46, G 1549 49), cell-division factors, tumour inhibitors (*eg* hadacidine 44) or antibiotic antagonists (*eg* ferrichromes 42). Although the particular physiological activity cannot be assigned in each case to the hydroxamic acid moiety, it is the outstanding chemical feature of these molecules, and it may be expected to play a role in the biological action of molecules of which it is a part.

It has been established that the hydroxamic acids are intimately associated with iron-transport phenomena[83,84] in those cases where they act as growth factors. Their synthesis can be precisely regulated. In severe iron deficiency a greater amount of these powerful sequestering agents for the very insoluble ferric ion is produced and excreted into the medium in massive amounts to scavenge the metal from inaccessible sources in the environment. Meanwhile, a highly specific transport system, localized in the cell membrane, protects the cell against the penetration of toxic quantities of inorganic iron and rejects the ligand unless it is laden with its specific metal ion. In the cell the ferric ion is probably reduced to the ferrous ion, which subsequently can be

delivered at the point of demand by the biosynthetic machinery of the cell.[12] This assumption is based on the knowledge that the iron(II) complexes are very unstable[15] and so provide a release mechanism for the iron. It has been proven that siderophore-mediated iron incorporation into microbial cells involves highly specific recognition by receptors at the cell surfaces.[14,85,86] The metal center and adjacent functionalities seem to be the key for the recognition by the receptor. Since for a number of bacteria the specific outer membrane receptor for ferrichrome also serves as a common binding site for some phages,[85] it has been suggested by Raymond[14] that ferrichrome-competing phages possess some structural features similar to the hydroxamate functionality of the ferrichrome molecule (42). A striking property of the ferric hydroxamate growth factors is their power to antagonize the toxicity of the related antibiotics.[12,13] Presumably the mechanism is one of competition for the same transport system, because further investigation has revealed close chemical similarity of the iron-binding site between both antibiotic and its antagonist.[13]

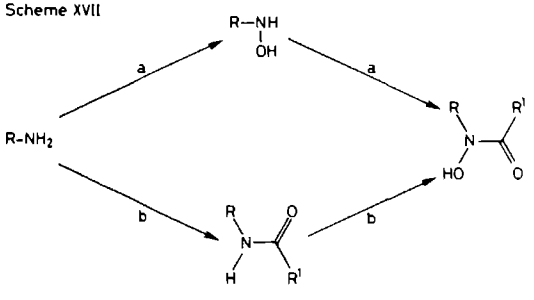
In the past the hydroxamic acids were divided into two classes according to their biological activity.[12] Those with growth-promoting activity were designated as sideramines and those with antibiotic properties as sideromycins. This terminology is not entirely satisfactory, since a number of the compounds is neither growth-factor nor antibiotic. Further, some of the antibiotics can serve as growth-factors depending on the test-organism. Therefore, this classification has been abandoned and the term siderophore (or, depending on the author(s), siderochrome) was retained for all microbial iron transport factors.

Finally, it is worth mentioning that several synthetic and naturally occurring hydroxamic acids have already been used as therapeutic agents in medicine to combat accidental metal poisoning.[14,87] In this way iron has been removed for example from iron-overloaded mice and humans.[14,87]

2.4 *BIOSYNTHESIS OF HYDROXAMIC ACIDS*

Inspection of the formulae of hydroxamic acids suggests that there are only two possible routes of biosynthesis that merit consideration; hydroxylation of the nitrogen atom may occur either before (pathway a, scheme XVII) or after (pathway b, scheme XVII) acylation.[12,88,89]

Scheme XVII



Whereas there is no chemical precedent for the direct oxidation of amino acids to N-hydroxy-α-amino acids, the biosynthesis of hadacidine 44 seems to proceed from the parent amino acid with an N-hydroxyamino acid as the first intermediate (pathway a).[88] Although free N-hydroxyamino acids have not yet been isolated from any hydroxamate producing organism, strong evidence for their involvement has been obtained. Molecular oxygen was found to be the source of the oxygen in N-hydroxyglycine,[88] the direct precursor of hadacidin.

Several of the hydroxamic acids that contain the hydroxamate group within a heterocyclic ring are derived from the appropriate amino acids, *e.g.* mycelianamide 45 (tyrosine, alanine)[90], aspergillic acid 46 (leucine, isoleucine)[91] and pulcherriminic acid 47 (leucine).[92,93] In two cases (aspergillic acid, pulcherriminic acid) convincing evidence was provided that the oxidation of the ring nitrogen takes place by a specific enzymatic reaction after the cyclization has occurred (pathway b).[88] It remains to be seen whether this is also true in the case of mycelianamide.

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CHAPTER III

CHIRAL HYDROXAMIC ACIDS AS LIGANDS IN ASYMMETRIC, METAL-CATALYZED REACTIONS

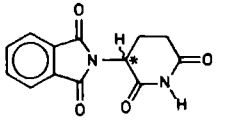
CHIRAL HYDROXAMIC ACIDS AS LIGANDS IN ASYMMETRIC, METAL-CATALYZED REACTIONS

3.1 *INTRODUCTION*

Molecules that are not superimposable on their mirror images are chiral.[1-3] Based on the Greek word for "hand" (cheir), chirality means "handedness", in reference to that pair of non-superimposable mirror images we constantly have before us: our two hands.

The organic constituents of plants, animals and microorganisms- from the most primitive to the most highly developed- are composed of molecules that are chiral. Life processes on a molecular scale take place between chiral molecules in a chiral environment. Living organisms, as a consequence of their chiral nature, exhibit stereoselection (*i.e.* chiral discrimination) in their interactions with chiral substances.[4]

Thus, the effect of biologically active substances (pharmaceuticals, agrochemicals), containing one or more chiral centres, sometimes changes drastically with the chirality of the molecules.[5-7,96] One of the most dramatic examples of this change concerns the drug thalidomide (softenon),[8] a hypnotic which was marketed in the sixties as a racemic mixture. It was withdrawn from



Thalidomide

the market because its administration was associated with fetal abnormalities. In 1979 it was discovered that the hypnotic action

has to be attributed to the enantiomer with the R-configuration, whereas the teratogenicity (ability to cause birth defects) of the drug is due solely to the S-enantiomer.[8,9] In spite of this knowledge most synthetic chiral drugs are still marketed as racemates or diastereomeric mixtures.[7] However, the situation is rapidly changing. The ever increasing demand for more selective drugs, insecticides etc., which show less toxic side-effects and are more environmentally acceptable is providing an important stimulus to chemists to prepare these compounds as optically pure isomers.

The impressive advances that have been made in developing methods for the production of pure stereoisomers have been reviewed amply.[6,10-16] The use of chiral reagents in catalytic quantities (*i.e.* chiral catalysis) offers one of the most promising approaches. Chiral catalysis[17] is a synthetic method with great potential because, in the best examples, a large amount of the chiral product can be made with only a small investment of chiral material in the catalytic system. That is why chiral catalysts are becoming very important at a time when the chemical industry is seeking ways of conserving energy and of making the best possible use of available resources.

Depending on the nature of the chiral catalyst one can distinguish between bio- and chemocatalysis. In the first case (immobilized) enzymes or intact cells are used to exert catalytic activity.[18-20] Although biocatalysts often constitute perfect chiral reagents, this kind of catalysts cannot always be applied to obtain the desired pure stereoisomer. This is mainly caused by the fact that most biocatalysts can furnish only one of the possible

stereoisomeric products. Besides, sometimes the isolation and/or use of biocatalysts is a demanding task and some of them display a limited substrate specificity.

Therefore chemocatalysis, *i.e.* the use of relatively simple chiral compounds (nonenzymic) from natural or synthetic origin as catalyst, receives much attention too. In this way it is tried to complement or improve the results achieved with biocatalysts. There are also many examples of chemical systems which mimic the action of enzymes (see e.g. ref. 21-27).

With respect to chemocatalysis the most innovative advances have been in the applications of metallo-organic chemistry in which the central metal atom along with one or more coordinated chiral ligands is used to guide and closely orient the stereochemical course of the reaction.[14,17] Examples of such reactions are the homogeneous hydrogenations catalyzed by rhodium-chiral phosphine complexes,[17] the peroxidic allyl alcohol oxidations catalyzed by titanium alkoxide and diethyl tartrate,[17,28] the Ni(II)-catalyzed Grignard cross-coupling reactions,[17,29] aldol condensations catalyzed by chiral Zn-complexes,[30] and several other reactions like cyclopropanation with carbenes,[31] the allylation of carbon nucleophiles,[17,32] Diels-Alder reactions,[33] hydrosilylations, hydrocarbonylations and isomerizations.[14,17]

The subtlety of the effects responsible for the asymmetric induction, *i.e.* the electronic and steric interactions in the transition state, makes it very difficult to design chiral inducing ligands, which are generally applicable. In this respect we became intrigued by the potential usefulness of chiral hydroxamic acids as ligands in asymmetric, metal-catalyzed reactions. Hydroxamic

acids form stable complexes with a large number of transition metals,[34-36] a property through which they are potentially applicable in various types of reactions. Although hydroxamic acids, which are usually cheap and readily accessible, have been applied as chiral ligands in some cases,[70-72] the chiral induction by this class of compounds has never been investigated systematically.

So, our aim was to investigate chiral hydroxamic acids for their ability to induce chirality in various transition metal-catalyzed reactions. In this chapter the synthesis of a few chiral hydroxamic acids is described. Subsequently, the results of their application as chiral ligands in the transition metal-catalyzed epoxidation of allylic alcohols (Sharpless-epoxidation),[17,28] a reaction which we regard as a touchstone for the viability of our approach, are reported.

3.2 *SYNTHESIS OF CHIRAL N-HYDROXY-DIOXOPIPERAZINES*

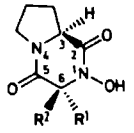
3.2.1 *Introduction*

The first thing to be done was taking a decision with respect to the structure of the hydroxamic acids to be synthesized. Although it is difficult to predict whether a certain compound will be capable of exerting an efficient chiral induction, it is not hard to imagine the primary conditions which have to be met in order to make a compound an attractive chiral ligand. Kagan[37] has summed up these criteria for the synthesis and use of a chiral ligand :

1. It must be coordinated to the metal during the step in which the chiral center on the substrate is created, and not exert merely a chiral medium effect.

2. The catalytic activity when the chiral ligand is present should be reasonably good relative to that of the achiral catalyst.
3. The structure of the ligand should allow for various chemical modifications to be made in order to permit the synthesis of variants. In this way optimal ligand-substrate matches can be sought.
4. The synthesis of the ligand must be relatively easy. If possible, resolution is to be avoided, the starting material being a cheap one taken from the "chiral pool"
5. It is desirable to be able to get both antipodes of the ligand.

We felt that hydroxamic acids of general structure 1 might satisfy these conditions. The bicyclic structure was settled upon



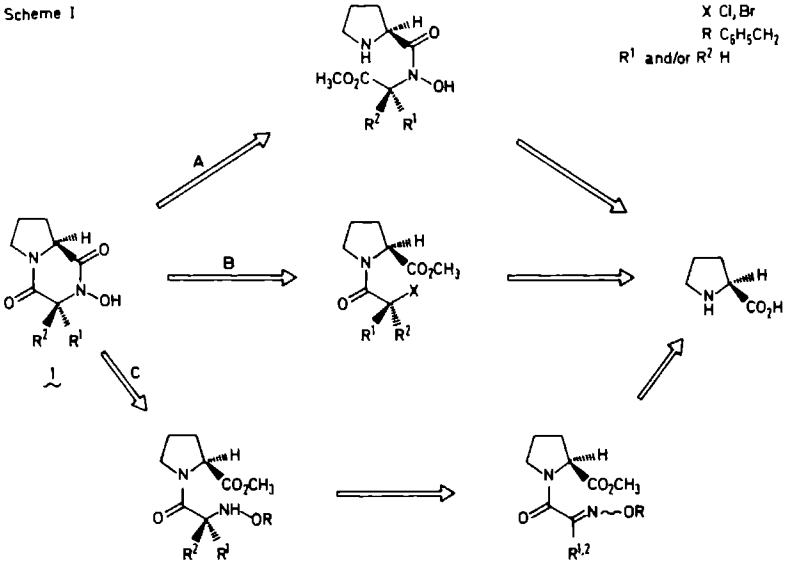
1

because of its rigidity. This feature and the bidentate nature of hydroxamic acids[34,38] should minimize the number of conformations the ligand can assume in the coordination sphere of the metal during the transition state. We intended to investigate the influence of the substituents R^1 and R^2, at least one of them being a hydrogen atom, and of the chirality at C_6 on the inductive power of 1.

There are several methods for synthesizing N-hydroxy-dioxopiperazines like 1.[39-44] In scheme I some possible retrosynthetic pathways for 1 are depicted.

It is clear that the target molecule is an N-hydroxy-dioxopiperazine, *i.e.* a cyclic N-hydroxy dipeptide, of (S)-proline[45] and an N-hydroxy-α-amino acid. We decided to prepare most of the

Scheme I

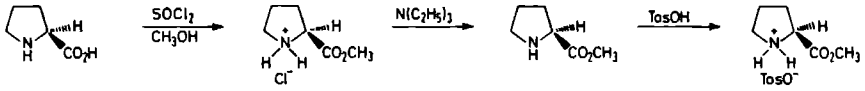


contemplated compounds, R^1 or R^2 being H, CH_3, i-C_3H_7 or C_6H_5, according to route B, since this seemed to be the most straightforward and cheapest way. In only one case, R^1 or R^2 being a benzyl group, route C was applied because the relevant α-halocarboxylic acid, needed for a synthesis according to route B, was not commercially available.

3.2.2 *Synthesis of N-(2-halo-acyl)-(S)-proline methyl esters*

Thus, (S)-proline 2 was converted into the corresponding methyl ester,[46] which was isolated as its HCl-salt 3 (scheme II). This

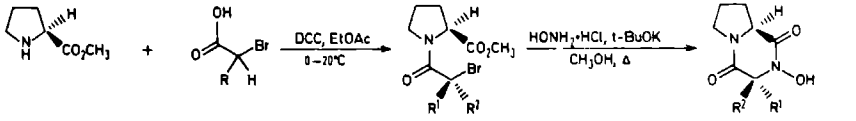
Scheme II



2 3 4 5

salt 3 is rather difficult to handle due to its hygroscopic nature. The free amine 4 could be obtained as an oil by treatment of 3 with triethylamine. In most cases 4 was used directly in a coupling reaction with an α-halocarboxylic acid (see scheme III); otherwise it was converted into the stable and less hygroscopic 4-toluenesulfonic acid salt 5.

Scheme III



4

6 R=H
7 R=CH₃
8 R=i-C₃H₇
9 R=C₆H₅

10 R¹=R²=H
11a R¹=CH₃, R²=H
b R¹=H, R²=CH₃
12a R¹=i-C₃H₇, R²=H
b R¹=H, R²=i-C₃H₇
13a R¹=C₆H₅, R²=H
b R¹=H, R²=C₆H₅

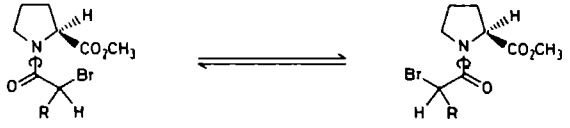
14 R¹=R²=H
15a R¹=CH₃, R²=H
b R¹=H, R²=CH₃
16a R¹=i-C₃H₇, R²=H
b R¹=H, R²=i-C₃H₇
17a R¹=C₆H₅, R²=H
b R¹=H, R²=C₆H₅

Scheme III shows how we tried to synthesize each of the various chiral hydroxamic acids. The first step involved a coupling of (S)-proline methyl ester 4 with an α-halocarboxylic acid, the formation of the amide bond being promoted by the coupling agent dicyclohexylcarbodiimide (DCC).[47] Subsequently, ring closure was effected by treatment of the resulting products (10-13) with an excess of hydroxylamine in refluxing methanol. Since the applied chiral α-halocarboxylic acids (7-9) were all optically inactive, the products consisted of two diastereomers. Theoretically the separation of the diastereomers could be carried out at any stage of the synthesis, *i.e.* after the coupling reaction or after the ring closure. In this respect we were confronted with a number of problems. In the following part of this section the specific properties of the

compounds, which underlay these problems, will be discussed in some detail.

Compound 10 was obtained easily in 62% yield, based on 4. The synthesis of 11 afforded a mixture of diastereomers, which could not be separated, in 54% yield. The diastereomeric ratio was approximately 2.4:1 as established by ^{1}H-NMR spectroscopy using the shift reagent Eu(fod)$_3$. The chromatographic separation of the diastereomers of 12 proved to be feasible, furnishing the less polar diastereomer 12a in 33% yield and the other, more polar one 12b in 23% yield.[48] The ^{1}H-NMR spectrum of the latter showed 8 peaks for the two diastereotopic methyl groups and 2 peaks for the methyl ester function, a phenomenon which must be attributed to the existence of two rotamers (*trans* and *cis*, ratio 2:1; see fig. 1).

Fig. 1



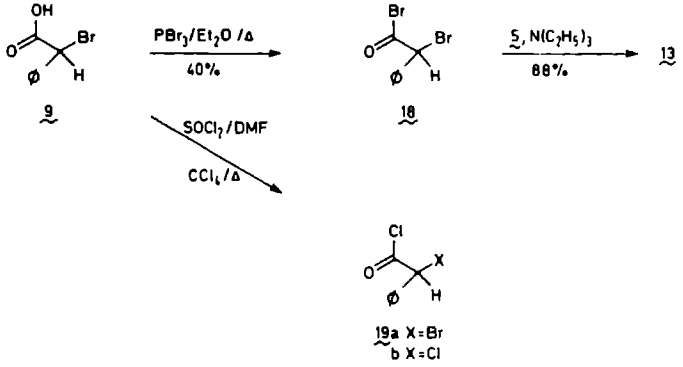
cis trans

It is well-known that hindered internal rotation of amide bonds can lead to the occurrence of distinct rotamers (*cis* and *trans*), which can be detected by ^{1}H-NMR spectroscopy if their interconversion proceeds at a rate which is slow on the NMR time scale.[49,50] Whereas most peptide bonds exist exclusively in the *trans* form, especially proline-derived amides or peptides often show this peculiar behaviour.[51] For the compound in question the coalescence temperature, *i.e.* the temperature at which the interconversion is so fast that the ^{1}H-NMR signals belonging to the different rotamers

coalesce completely,[49] was found to be approximately 70°C. This phenomenon was observed only with the more polar diastereomer of 12 (12b). From the ^{1}H-NMR spectra of 10, 11 and the less polar diastereomer of 12 (12a) it could not be deduced whether distinct rotamers exist in solution at room temperature.

For the synthesis of compound 13 two different methods for the activation of the carboxylic acid function of 9 were applied. At first we synthesized 13 in 88% yield by treatment of the 4-toluenesulfonic acid salt 5 of (S)-proline methyl ester with the acid bromide 18 in the presence of triethylamine (Scheme IV). The acid bromide 18 was readily accessible by treatment of 9 with PBr_3, the yield being 40%.

Scheme IV

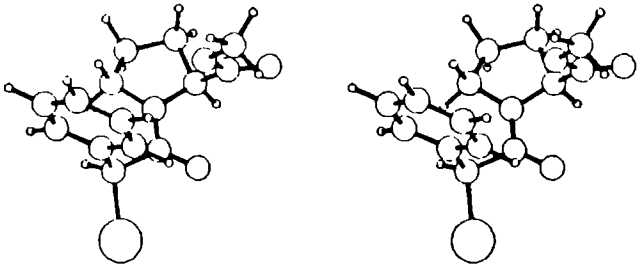


Compound 18 had been chosen because all attempts to prepare the acid chloride 19a failed due to partial or complete substitution of the benzylic bromine by chlorine, resulting in the formation of a mixture of 19a and 19b resp. pure 19b. The formation of 13 proceeded smoothly provided that the reaction mixture did not contain any chloride ions, since compound 13 was also prone to

halide exchange.

Unfortunately, we were confronted with a problem concerning the reproducibility of the results. While the yield remained unchanged, the ratio of diastereomers was different each experiment. It varied from nearly 50:50 to 100:0, the more polar diastereomer always being the major product. By means of X-ray diffraction[52] it was established that both chiral centres of the latter compound had the S-configuration. Thus structure 13a (see also fig. 2) could be assigned to this compound.

Fig. 2 Stereoview of 13a



It was impossible to obtain the less polar diastereomer 13b as a pure compound by column chromatographic separation of 13a and 13b; at best a mixture enriched in 13b was obtained.

3.2.3 *Crystallization-induced asymmetric transformation of N-(2-bromo-2-phenyl-acetyl)-(S)-proline methyl ester*

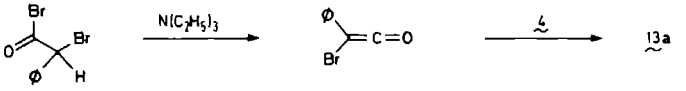
Attempts to find an explanation for the varying product ratios in the synthesis of 13 were hampered by the complexity of the ^{1}H-NMR spectrum. From the presence of two peaks for the benzylic hydrogen atom and the methyl ester function of each diastereomer it was deduced that distinct rotamers of 13a and 13b were present in solution. This conclusion was supported by the coalescence of the

relevant signals of both diastereomers at approximately 75°C.

Several explanations for the incidental formation of one pure diastereomer were considered. It was demonstrated that the product formation was not the result of a thermodynamically controlled epimerization,[53] which could have been caused by the presence of a small excess of triethylamine in some of the reaction mixtures.

Another possibility, involving the transient formation of ketene 20 followed by asymmetric nucleophilic addition[54] of 4 to 20 (eq.1), could also be ruled out, among others by the absence of

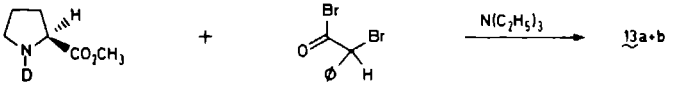
(eq 1)



18 20

deuterium in the product obtained by coupling 18 with deuterated proline methyl ester 4.D (eq.2).[55]

(eq 2)



4.D 18

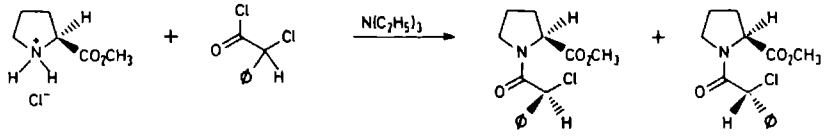
We perceived that the variation of the diastereomeric ratio also occurred when the coupling reaction was accomplished by applying the coupling reagent DCC (Scheme III; yield 13a/b : 59%). The results of these experiments ruled out the possibility that the observed product formation could be explained by the intermediacy of 20. Nevertheless, the last two experiments put us on the right scent.

We found that dissolution of pure 13a or any mixture of 13a and 13b in *e.g.* $CDCl_3$, CH_2Cl_2, CD_3OD or EtOAc always resulted ultimately in the 'spontaneous' formation of a 50:50 mixture, but the

equilibrium was reached quite slowly. Evaporation of the solvent led to a crystallization-induced asymmetric transformation[56]; crystallization of pure 13a shifted the equilibrium of the diastereomeric mixture (13a/b) completely towards precipitation of 13a.

It was not investigated in detail whether the asymmetric transformation was caused by C-H or C-Br bond dissociation. Since the chlorine analogue 21, prepared from 3 and 19b (eq.3),[57] did not

(eq 3)



3 19b 21a 22% 21b 28%

undergo this asymmetric transformation and since equilibration of 13 in CD_3OD gave no incorporation of deuterium at all, we are inclined to favour the latter assumption, *i.e.* C-Br bond dissociation.

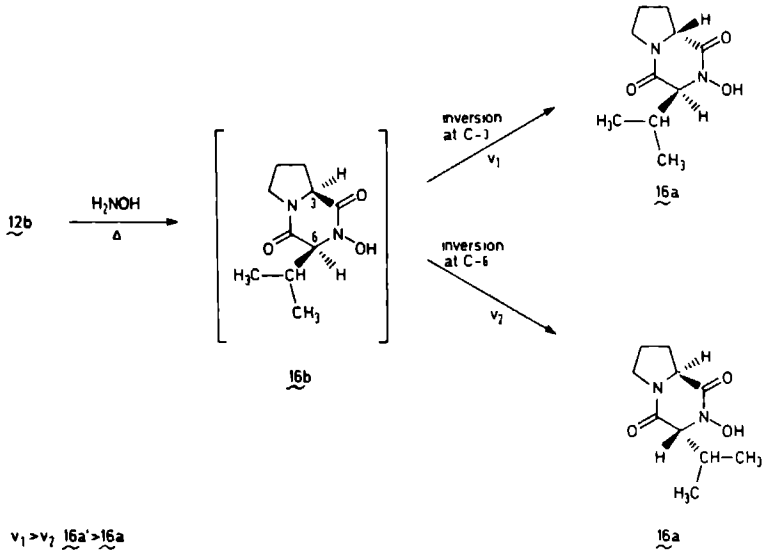
3.2.4 *Ring closure*

The final step in the synthesis of the chiral ligands involved ring closure of the N-(2-halo-acyl)-(S)-proline methyl esters 10-13 (and 21) by treatment with an excess of hydroxylamine (scheme III). Compound 14 was obtained from 10 in 25% yield after recrystallization. It is obvious that the conversion of the mixture 11a/b into 15 gave a mixture of diastereomers. Although it was impossible to distinguish between the diastereomers of 15 on TLC, one pure diastereomer could be isolated in 46% yield by careful column chromatography; structure 15b was assigned tentatively to this compound.[58] Besides, the separation procedure furnished a mixture enriched in the other diastereomer 15a (ratio 4.5:1) in 6% yield.

The products obtained after ring closure of 12a (61% yield) and

12b (20% yield) were identical in all respects with the exception of the specific rotation. In view of the results produced with the compounds 13a and 21b (*vide infra*) we reasoned that 12a was converted into 16a and that the primary product 16b formed from 12b epimerized under the reaction conditions to the thermodynamically more stable compounds 16a/16a' (Scheme V).

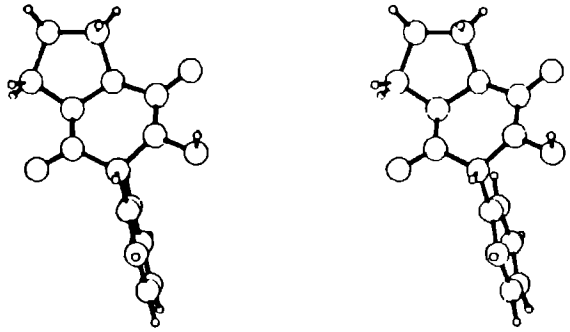
Scheme V



The specific rotation of the mixture 16a/16a' indicated that in 16b both chiral centres are liable, though not to the same degree, to proton abstraction.

Compound 17a was readily accessible by ring closure of 13a or 21a, the former giving the best yield, viz. 75%. The absolute configuration of the ring-closed product 17a was determined by X-ray analysis[59] (fig.3). Thus, it was proved that substitution of the halo atom proceeds stereospecifically according to an S_N2 process.

Fig.3 Stereoview of 17a



A mixture of diastereomers (17a/b) was obtained when a mixture of 13a/b or pure 21b was used as starting material. In the latter case 17b could be isolated in 14% yield. It was shown that this compound epimerized under the reaction conditions to 17a (eq.4);

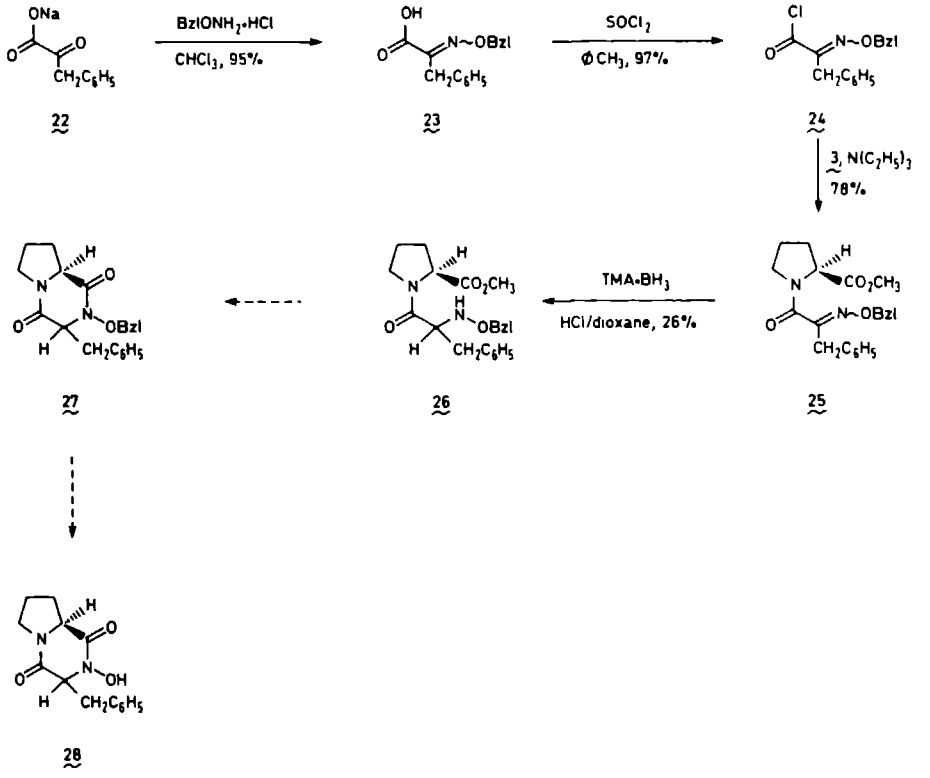
(eq 4) 17b $\underset{\text{X}}{\overset{H_2NOH/CH_3OH/\Delta}{\rightleftharpoons}}$ 17a

compound 17a is thermodynamically more stable than 17b, presumably because of the '*trans*' orientation of the bulky substituents at C_3 and C_6. Since the C_6-(benzylic) proton is much more acidic than the C_3-proton, inversion proceeded probably only at the benzylic chiral centre.

3.2.5 *Attempted synthesis of cyclo-[(S)-prolyl-N-hydroxyphenylalanyl]*

Unfortunately, all attempts to prepare 28 failed (Scheme VI). Whereas the synthesis of 25 caused no problems, the reduction of 25 to 26, an interesting reaction because of the possibility of asymmetric induction, proceeded quite slowly despite of the presence of a large excess of reducing agent. The ^{1}H-NMR spectrum of 26 was rather complicated and gave no clear indication for the

Scheme VI



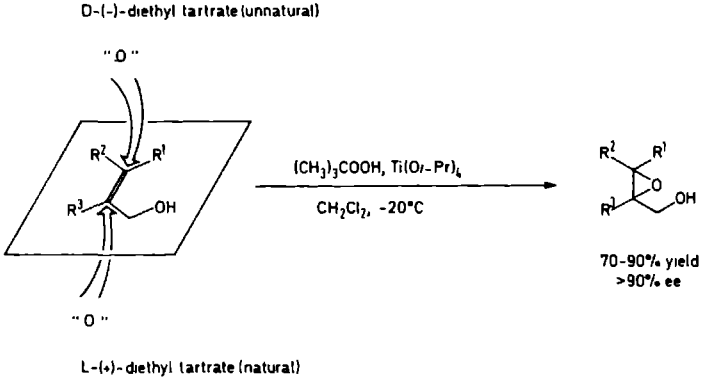
presence of diastereomers and/or rotamers. Some preliminary attempts to cyclize 26 to 27 were not successful.

3.3 *TRANSITION METAL-CATALYZED ASYMMETRIC EPOXIDATION OF ALLYLIC ALCOHOLS : A TOUCHSTONE*

The following objective was to evaluate the chiral inducing ability of the hydroxamic acids 14-17 in the well-studied transition metal-catalyzed epoxidation of allylic alcohols. This type of reaction has attracted much attention during the last decade,

mainly because of the development by K.B. Sharpless[60,61] of a catalytic system with overwhelming intrinsic prochiral face selectivity. His invention involves the use of catalytic amounts of titanium(IV) isopropoxide and diethyl(+)- or (-)-tartrate to effect a stereoselective reaction of tert-butyl hydroperoxide with allylic alcohols (eq.5).

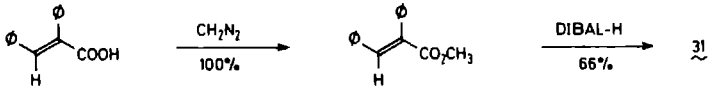
(eq 5)



The unique combination of reagents provides consistently the enantiofacial selection shown in eq. 5, regardless of the substitution pattern of the olefin. The scope and mechanism of the so-called Sharpless-epoxidation have been reviewed amply.[17,28,62,63]

In order to find the best, catalytically active hydroxamic acid-metal complex we intended to vary both the metal ion and the structure of the hydroxamic acid. The compounds 29,[64] 30 and 31 served as substrates (see eq.7), 31 being prepared in a two-step procedure from (E)-α-phenylcinnamic acid 32 (eq.6).

(eq 6)

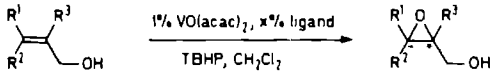


32 33

First of all the proper reaction conditions were established using 17a as chiral ligand. The results of these preliminary

experiments are summarized in eq.7/table 1.

(eq 7)



29 $R^1 = n\text{-}C_6H_9$, $R^2 = R^3 = H$ 34

30 $R^1 = (CH_3)_2C=CHCH_2CH_2$, $R^2 = CH_3$, $R^3 = H$ 35

31 $R^1 = R^3 = C_6H_5$, $R^2 = H$ 36

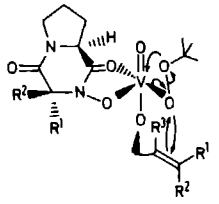
Table 1

Substrate	x	ligand	temp., °C	yield (%)	e.e.(%)[65,66]
29	3	17a	0	77 [67]	38
30	3	17a	-20 → +20	56 [68]	42 (2S,3S)
30	3	17a	-20 → +20	47 [69]	43 (2S,3S)
30	3	17a	20	89	53 (2S,3S)
30	5	17a	20	75	57 (2S,3S)
30	3	17a	0	75	62 (2S,3S)
30	5	17a	0	17	66 (2S,3S)
31	5	17a	0 → +20	72	55

The results show that the stereoselectivity increases as the temperature decreases and more equivalents of hydroxamic acid, with respect to vanadyl acetylacetonate, are used. The latter aspect deserves some further comment.

It is assumed[70,71] that the hydroxamic acid forms a complex with a vanadium(V)-species, which arises from the vanadium(IV)-catalyst by tert-butyl hydroperoxide-mediated oxidation. In accordance with earlier proposals[62,70,73] we think that complex 37

may be involved as an intermediate in the reaction.



37

Probably, the complex is in equilibrium with its constituents, the excess of hydroxamic acid being necessary to shift the equilibrium. In this way the amount of vanadium species bearing no hydroxamate ligand is reduced to a minimum; the presence of such a species must be avoided since it would catalyze the formation of racemic epoxy alcohol.

Typically our reactions proceeded much slower than the corresponding conversions accomplished with the Sharpless catalyst.[61,63] Besides, the yield was lower as a longer reaction time was required for complete conversion. This made it unattractive to carry out the reaction at 0°C and/or in the presence of 5 equivalents of hydroxamic acid.

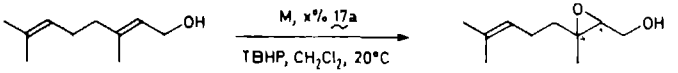
The results obtained with the other hydroxamic acids are depicted in table 2 (see also eq.7). From the data it may be concluded that the stereoselectivity is favoured by the presence of a bulky substituent at C_6 in a *trans* orientation with respect to the proline ring (see structure 1). When the substituent and the proline ring are on the same side of the dioxopiperazine ring (*cis*-configuration, *i.e.* 15b and 17b) only a poor chiral induction is observed.

Table 2

Substrate	x	ligand	temp., °C	yield(%)	e.e.(%)[65,66]
30	3	14	20	76	23 (2S,3S)
30	3	15b [74]	20	65	13 (2S,3S)
30	3	16a [74]	20	73	50 (2S,3S)
30	3	16a/16a'[74]	20	76	38 (2R,3R)
30	3	17b [75a]	20	77	3 (2S,3S)
30	5	17b [75a]	20	79	7 (2S,3S)
31	3	14	20	64	17
31	3	15b [74]	20	76	4
31	3	16a [74]	20	85	57
31	5	17b [75a]	0 → 20	77	5 [75b]

Finally the influence of the metal ion on the reactivity and stereoselectivity was investigated briefly (eq.8/table 3).

(eq 8)



30 35

These reactions proceeded slower than the corresponding V-catalyzed epoxidations and the stereoselectivity was disappointingly low. Acquaintance with the formation (or stability) constants would have been helpful in determining the amount of hydroxamic acid needed for an optimum chiral induction. Unfortunately, there is not much information available on the complexation of hydroxamic acids with V, Mo or Ti.[78]

Table 3

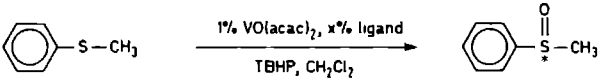
M	x	yield (%)	e.e. (%)[65,66]
1% $Mo(CO)_6$ [76]	1,1	64	10 (2S,3S)
1% $Mo(CO)_6$ [76]	3	33 [77]	11 (2S,3S)
10% $Ti(Oi\text{-}Pr)_4$	11	62 [77]	27 (2S,3S)
10% $Ti(Oi\text{-}Pr)_4$	21	60 [77]	28 (2S,3S)

3.4 *ASYMMETRIC OXIDATION OF SULFIDES*

A possible application we had in mind for the hydroxamic acid derived catalytic system concerned the asymmetric oxidation of sulfides to sulfoxides. The total synthesis of sparsomycin, a naturally occurring antibiotic and cytostatic, was one of the topics investigated by our group.[79] An approach involving the oxidation of deoxosparsomycin[80]-precursors with $NaIO_4$ was unsatisfactory, since predominantly undesired regioisomers were produced this way.[79a]

Searching for a method for the regio- and diastereoselective oxidation our attention was focussed on the use of TBHP in combination with a chiral catalyst, *i.e.* a chiral vanadyl-hydroxamate complex. To start with we selected thioanisole 38 as a simple model substrate to study the feasibility of the catalytic system. The results are summarized in eq.9/table 4.

(eq 9)



38 39

Table 4

x	ligand	temp., °C	yield (%)	$[\alpha]_D^{20}$*	opt. purity (%)[81,82]
3	17a	20	69	-9.6°	6.5 (S)
3	17a	0	83	-13.3°	9 (S)
5	17a	0	77	-16.1°	11 (S)
3	17b	0	83	+4.1°	3 (R)

* c=1.7; acetone

Only poor chiral inductions were realized. Besides, it turned out to be impossible to accomplish complete conversions. A rationale for this failure might be the following. Probably the sulfoxide 39 has a higher affinity for the metal ion and thus competes with the starting material for a position in the metal complex. Our observations were confirmed by some literature reports[81b,83,84] describing that the reaction slows down and the optical yield lowers considerably when the amount of catalyst is reduced.

3.5 *CONCLUSIONS*

The preliminary results reported in this chapter are encouraging. Since the choice of the ligand is quite empirical, further research concerning the structure of the catalytically active metal complex will be necessary. To gain more insight into the influence of steric and electronic interactions on the stereochemical course of the reaction, additional variations in the ligand have to be considered. In this way an optimal ligand-substrate match, leading to improvement of the stereoselectivity without retarding the conversion too much, may be found.

Concerning the asymmetric oxidation of sulfides we conclude that the applied method, employing merely a catalytic amount of V^{5+}, is not feasible for the synthesis of chiral sulfoxides of high optical purity.

3.6 *EXPERIMENTAL SECTION*

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Infrared spectra were measured with a Perkin-Elmer Model 397 spectrophotometer. Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as the solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer.

Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with an UV hand lamp, iodine vapor or Cl_2-TDM.[85] Hydroxamic acids were detected (red spots) by spraying with a 3% $FeCl_3$ (w/v) solution in concentrated HCl/MeOH (4:96, v/v). The allylic alcohols (29-31), the epoxy alcohols (34-36) and epoxy acetates were visualized by spraying with 50% H_2SO_4/MeOH (v/v), followed by heating.

For column chromatography Merck silica gel H (type 60) was used. The Miniprep LC (Jobin Yvon) was used for preparative HPLC. Solvent systems used were as follows : system A, MeOH/CH_2Cl_2 (2:98, v/v); system B, EtOAc/n-hexane (35:65, v/v); system C, EtOAc/n-hexane (1:4, v/v); system D, MeOH/CH_2Cl_2 (7:93, v/v); system E, MeOH/$CHCl_3$

(7:93, v/v); system F, MeOH/CH_2Cl_2 (4:96, v/v); system G, MeOH/CH_2Cl_2 (3:97, v/v); system H, CH_2Cl_2; system I, EtOAc/n-hexane (30:70, v/v); system J, EtOAc/n-hexane (1:1, v/v); system K, EtOAc/n-hexane (3:1, v/v).

The α-halocarboxylic acids 6-9, geraniol 30, (E)-α-phenylcinnamic acid 32, and 70% aqueous TBHP were purchased from Janssen Chimica or Aldrich, Benelux.

(S)-Proline methyl ester hydrochloride salt 3

This compound was prepared from (S)-proline (Janssen Chimica) according to the procedure described by St. Guttmann.[46] $[\alpha]_D^{20}$: -33.2° (c=1.1; H_2O), lit.[46] $[\alpha]_D^{24}$: -34.0° (c=1.1; H_2O); ^{1}H-NMR (D_2O; t-BuOH) : δ 4.49 (m, 1H, $CHCO_2$), 3.83 (s, 3H, CO_2CH_3), 3.44 (m, 2H, CH_2N), 2.65-1.82 (m, 4H, $CH_2CH_2CH_2N$); ($CDCl_3$/TMS) : δ 4.52 (m, 1H, $CHCO_2$), 3.83 (s, 3H, CO_2CH_3) 3.55 (m, 2H, CH_2N), 2.69-1.78 (m, 4H, $CH_2CH_2CH_2N$).

(S)-Proline methyl ester 4

Triethylamine (5.00 g, 49.5 mmol) was added dropwise to a stirred and chilled (0°C) solution of 3 (8.27 g, 50 mmol) in 50 ml of dry CH_2Cl_2. After completion of the addition the mixture was stirred at room temperature for 30 minutes. The solvent was evaporated in vacuo after which the resulting residue was washed thoroughly with Et_2O (4 x 100 ml). The ethereal solutions obtained after filtration were combined and concentrated in vacuo, thus affording proline methyl ester 4 as an oil in 72% yield[86]; ^{1}H-NMR : δ 3.71 (m and s, 4H, $CHCO_2CH_3$), 3.21-2.65 (m, 2H, CH_2N), 2.31 (s, 1H, NH), 2.22-1.45 (m, 4H, $CH_2CH_2CH_2N$).

(S)-Proline methyl ester 4-toluenesulfonic acid salt 5

(S)-proline methyl ester 4 (4.64 g, 36 mmol) was dissolved in 50 ml of CH_2Cl_2 and treated with a solution of 4-toluenesulfonic acid monohydrate (6.84 g, 36 mmol) in 100 ml of CH_2Cl_2. After stirring at room temperature for 30 minutes the solution was dried over Na_2SO_4 and concentrated to dryness in vacuo. Recrystallization of the residue from CH_2Cl_2/n-hexane afforded 5 as a white solid in 82% yield based on 4 : thus the overall yield of 5 from 3 was 59%; ^{1}H-NMR : δ 8.87 (br.s., 2H, NH_2), 7.70 and 7.13 (AA'BB', 4H, C_6H_4), 4.53 (m, 1H, $CHCO_2$), 3.72 (s, 3H, CO_2CH_3), 3.47 (m, 2H, CH_2N), 2.56-1.68 (m, 4H, $CH_2CH_2CH_2N$), 2.37 (s, 3H, $CH_3C_6H_4$); chemical-ionization mass spectrum, m/e (relative intensity) : 173 ($[CH_3C_6H_4SO_3H + 1]^+$, 26%), 130 ($[4 + 1]^+$, 72%), 98 ($[4 - OCH_3]^+$, 15%), 70 ($[4 - CO_2CH_3]^+$, 100%); anal. calcd. for $C_{13}H_{19}NO_5S$: C, 51.81; H, 6.36; N, 4.65, found : C, 51.96; H, 6.36; N, 4.65.

General procedure for the synthesis of methyl N-(2-bromo-acyl)-(S)-prolinates 10-13

To a chilled (0°C) solution of proline methyl ester 4 (5.16 g, 40 mmol) in 100 ml of EtOAc (10-12) or CH_2Cl_2 (13) 44 mmol of the relevant α-halocarboxylic acid (6, 7, 8 or 9) was added. Subsequently, DCC (9.06 g, 44 mmol) was added all at once to the clear solution, which was stirred at 0°C for 15 minutes and at room temperature overnight. The precipitate (dicyclohexylurea) was then removed by filtration and the filtrate was concentrated to dryness in vacuo. The residue was purified by column chromatography (solvent system A for 10 and 12; solvent system B for 11 and 13).

N-(2-Bromo-acetyl)-(S)-proline methyl ester 10 was obtained as an oil, which solidified upon prolonged storage in a refrigerator, in 62% yield from 4. The compound was homogeneous on TLC (Rf 0.09, solvent system B) and could be recrystallized from EtOAc, m.p. : 42-44°C; ^{1}H-NMR : δ 4.69-4.39 (m, 1H, $CHCO_2$), 3.84 (s, 2H, CH_2Br), 3.84-3.50 (m, 2H, CH_2N), 3.73 (s, 3H, CO_2CH_3), 2.46-1.74 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); exact mass calcd. for $C_8H_{13}BrNO_3$ (M^++1), m/e 250.0079, found : 250.0079; chemical-ionization mass spectrum, m/e (relative intensity) : 252/250 ($[M+1]^+$, 63%) 220/218 ($[M-OCH_3]^+$, 20%), 192/190 ($[M-CO_2CH_3]^+$, 100%), 172 ($[M+2H-Br]^+$, 2%), 170 ($[M-Br]^+$, 18%), 142 ($[M-Br-CO]^+$, 4%), 130 ($[M+1-BrCHCO]^+$, 3%), 128 ($[M-BrCH_2C(O)]^+$, 5%), 112 ($[M+1-Br-CO_2CH_3]^+$, 2%), 70 ($[C_4H_8N]^+$, 23%); anal. calcd. for $C_8H_{12}BrNO_3$: C, 38.42; H, 4.84; N, 5.60, found : C, 38.15; H, 4.80; N, 5.53.

N-[(R,S)-2-Bromopropionyl]-(S)-proline methyl ester 11 was obtained as a mixture of diastereomers (yield : 54%), the ratio being approximately 2.4:1 as established by ^{1}H-NMR spectroscopy using the shift reagent tris-(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium, $Eu(fod)_3$. The product was homogeneous on TLC (Rf 0.16, solvent system B) and could be recrystallized from EtOAc, m.p. : 125-129°C; ^{1}H-NMR : δ 4.71-4.35 (m, 1H, $CHCO_2$), 4.43 and 4.21 (2 x q, 1H, CHBr), 4.00-3.33 (m, 2H, CH_2N), 3.75 and 3.71 (2 x s, 3H, CO_2CH_3), 2.43-1.92 and 1.80 (m resp. d, 7H, $C\underline{H}_2C\underline{H}_2CH_2N$ and $CHBrC\underline{H}_3$); exact mass calcd. for $C_9H_{15}BrNO_3$ (M^++1), m/e 264.0235, found : 264.0233; chemical-ionization mass spectrum, m/e (relative intensity) : 266/264 ($[M+1]^+$, 70%), 234/232 ($[M-OCH_3]^+$, 18%), 206/204 ($[M-CO_2CH_3]^+$, 100%), 186 ($[M+2H-Br]^+$, 14%), 184 ($[M-Br]^+$, 39%), 156 ($[M-Br-CO]^+$, 26%), 130 ($[M+1-BrC(CH_3)CO]^+$, 7%),

128 ([M-BrCH(CH_3)C(O)]$^+$, 14%), 126 ([M+1-Br-CO_2CH_3]$^+$, 6%), 70 ([C_4H_8N]$^+$, 57%); anal. calcd. for $C_9H_{14}BrNO_3$: C, 40.92; H, 5.34, N, 5.30, found : C, 40.91; H, 5.40; N, 5.27.

N-[(R,S)-2-Bromo-3-methyl-butanoyl]-(S)-proline methyl ester 12: the procedure for the synthesis of 12 was unlike the other syntheses in that 1-hydroxybenzotriazole hydrate (6.73 g, 44 mmol) was added to the reaction mixture prior to the addition of DCC. The reaction mixture had to be warmed up temporarily to achieve complete dissolution of the 1-hydroxybenzotriazole. Subsequently, treatment with DCC took place at 0°C again. Purification of the crude product by column chromatography (solvent system A) afforded a mixture of diastereomers in 86% yield. Separation of the diastereomers was accomplished by column chromatography, applying solvent system C.

Compound 12a, *N-[(S)-2-Bromo-3-methyl-butanoyl]-(S)-proline methyl ester*,[48] was obtained in 33% yield. The compound was homogeneous on TLC (Rf 0.28, solvent system B) and was recrystallized from EtOAc/n-hexane, m.p.: 55-57°C; ^{1}H-NMR : δ 4.71-4.34 (m, 1H, $CHCO_2$), 4.06 (d, 1H, CHBr), 3.83-3.41 (m, 2H, CH_2N), 3.70 (s, 3H, CO_2CH_3), 2.60-1.80 (m, 5H, $C\underline{H}_2C\underline{H}_2CH_2N$ and $C\underline{H}(CH_3)_2$), 1.16 and 1.03 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{11}H_{19}BrNO_3$ (M^++1), m/e 292.0548, found : 292.0546; chemical-ionization mass spectrum, m/e (relative intensity) : 294/292 ([M+1]$^+$, 100%), 262/260 ([M-OCH_3]$^+$, 15%), 234/232 ([M-CO_2CH_3]$^+$, 75%), 214 ([M+2H-Br]$^+$, 61%), 212 ([M-Br]$^+$, 62%), 182 (11%), 154 ([M+1-Br-CO_2CH_3]$^+$, 21%), 130 ([M+1-Br(i-C_3H_7)CC(O)]$^+$, 45%), 128 ([M-Br(i-C_3H_7)CHC(O)]$^+$, 82%), 70 ([C_4H_8N]$^+$, 9%); anal calcd. for $C_{11}H_{18}BrNO_3$: C, 45.22; H, 6.21; N, 4.79, found : C, 44.40; H, 6.11; N, 4.69.

Compound 12b, *N-[(R)-2-bromo-3-methyl-butanoyl]-(S)-proline methyl ester,*[48] was obtained in 23% yield. The compound was homogeneous on TLC (Rf 0.25, solvent system B) and was recrystallized from EtOAc/n-hexane, m.p.: 74-76°C; ^{1}H-NMR: δ 4.65 -4.35 (m, 1H, $CHCO_2$), 4.00 (d, 1H, CHBr), 3.91-3.36 (m, 2H, CH_2N), 3.76 and 3.73 (2xs, 3H, CO_2CH_3), 2.62-1.74 (m, 5H, $C\underline{H}_2C\underline{H}_2CH_2N$ and $C\underline{H}(CH_3)_2$), 1.20-0.95 (8 lines, 6H, $(CH_3)_2$). The ratio of rotamers was estimated at 2:1 in $CDCl_3$. For the determination of the coalescence temperature (± 70°C) CD_3NO_2 (ratio rotamers 4:1) was used as solvent. Exact mass calcd. for $C_{11}H_{19}BrNO_3$ (M^++1), m/e 292.0548, found: 292.0543; chemical-ionization mass spectrum, m/e (relative intensity) : 363/361 ($[M+C_4H_8N]^+$, 9%), 294/292 ($[M+1]^+$, 70%), 262/260 ($[M-OCH_3]^+$, 10%), 234/232 ($[M-CO_2CH_3]^+$, 52%), 214 ($[M+2H-Br]^+$, 37%), 212 ($[M-Br]^+$, 60%), 182 (4%), 154 ($[M+1-Br-CO_2CH_3]^+$, 8%), 130 ($[M+1-Br(i-C_3H_7)CC(O)]^+$, 22%), 128 ($[M-Br(i-C_3H_7)CHCO]^+$, 48%), 70 ($[C_4H_8N]^+$, 100%); anal. calcd. for $C_{11}H_{18}BrNO_3$: C, 45.22; H, 6.21; N, 4.79, found: C, 45.10; H, 6.22; N, 4.77.

N-[(R,S)-2-Bromo-2-phenyl-acetyl]-(S)-proline methyl ester 13, which was obtained in 59% yield, was homogeneous on TLC (Rf 0.24, solvent system B), no matter what the diastereomeric composition was.

Compound 13a, *N-[(S)-2-bromo-2-phenyl-acetyl]-(S)-proline methyl ester,* could be recrystallized from EtOAc, m.p.: 137-139°C; $[\alpha]_D^{22}$: -2.5° (c=0.7; $CHCl_3$); ^{1}H-NMR : δ 7.62-7.27 (m, 5H, C_6H_5), 5.62 and 5.47 (2xs, 1H, CHBr), 4.70-4.45 and 4.45-4.16 (2xm, 1H, $CHCO_2$), 3.82 and 3.69 (2xs, 3H, CO_2CH_3), 3.79-3.33 (m, 2H, CH_2N), 2.35-1.74 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); exact mass calcd. for $C_{14}H_{17}BrNO_3$

(M^++1), m/e 326.0392, found: 326.0389; chemical-ionization mass spectrum, m/e (relative intensity) : 397/395 ($[M+C_4H_8N]^+$, 2%), 328/326 ($[M+1]^+$, 21%), 296/294 ($[M-OCH_3]^+$, 1%), 268/266 ($[M-CO_2CH_3]^+$, 6%), 248 ($[M+2H-Br]^+$, 14%), 246 ($[M-Br]^+$, 100%), 188 ($[M+1-Br-CO_2CH_3]^+$, 2%), 130 ($[M+1-C_6H_5C(Br)C(O)]^+$, 2%), 128 ($[M-C_6H_5CH(Br)CO]^+$, 22%), 70 ($[C_4H_8N]^+$, 12%); anal. calcd. for $C_{14}H_{16}BrNO_3$: C, 51.55; H, 4.94; N, 4.29, found : C, 51.58; H, 4.99; N, 4.27.

Equilibrium mixture of 13a and 13b (ratio 1:1); ^{1}H-NMR : δ 7.73-7.24 (m, 5H, C_6H_5), 5.60, 5.56, 5.46 and 5.36 (4xs, 1H, CHBr), 4.74-3.99 (m, 1H, $CHCO_2$), 3.87-3.32 (m, 2H, CH_2N), 3.80, 3.75, 3.68 and 3.57 (4xs, 3H, CO_2CH_3), 2.45-1.73 (m, 4H, $CH_2CH_2CH_2N$); the ratio of rotamers was 9:1 (13a) resp. 4.5:1 (13b). For the determination of the coalescence temperature (± 75°C for both diastereomers) CD_3NO_2 was used as solvent; in this solvent the ratio of rotamers was found to be 11:1 (13a) resp. 5.5:1 (13b).

(R,S)-2-Bromo-2-phenyl-acetyl bromide 18 (α-bromophenylacetyl bromide)

PBr_3 (171 g, 0.63 mol) was added all at once to a solution of 2-bromo-2-phenyl-acetic acid (32.44 g, 0.15 mol) in 100 ml of dry Et_2O. The reaction mixture was refluxed overnight. Then the solvent was removed by evaporation in vacuo, whereupon the pure product 18 was obtained in 40% yield by distillation of the residue under diminished pressure (b.p. 135°C; 5 mm Hg). The product solidified upon prolonged storage in a refrigerator; ^{1}H-NMR (60 MHz) : δ 7.36 (s, 5H, C_6H_5) and 5.69 (s, 1H, CHBr); IR (CCl_4) : 1810 cm^{-1} (C=O).

N-[(R,S)-2-Bromo-2-phenyl-acetyl]-(S)-proline methyl ester 13 from

(S)-proline methyl ester 4-toluenesulfonic acid salt 5 and 2-bromo-2-phenyl-acetyl bromide 18

Triethylamine (0.84 g, 8.4 mmol) was added dropwise to a chilled (0°C) solution of 5 (1.21 g, 4 mmol) and 18 (1.22 g, 4.4 mmol) in 4 ml of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 hours after which the solvent was evaporated in vacuo. Subsequently, the residue was suspended in EtOAc, and washed successively with 0.1N aqueous HCl (two clear layers were obtained), 5% aqueous $NaHCO_3$ (w/v) and brine. Finally the organic layer was dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded a residue which was purified by column chromatography as described before. Thus compound 13 was obtained in 88% yield.

N-[(R,S)-2-Chloro-2-phenyl-acetyl]-(S)-proline methyl ester 21

Triethylamine (4.66 g, 46 mmol) was added dropwise to a chilled (0°C) and stirred solution of 3 (3.64 g, 22 mmol) and 2-chloro-2-phenyl-acetyl chloride 19b (Janssen Chimica, 4.58 g, 24 mmol) in 50 ml of dry CH_2Cl_2. After completion of the addition, stirring was continued at room temperature for 4 hours. The product was isolated in 74% overall yield according to the same work-up procedure as described for 13. The diastereomers were separated by means of repeated column chromatography (solvent system B).

N-[(S)-2-Chloro-2-phenyl-acetyl]-(S)-proline methyl ester 21a[57] was obtained in 22% yield from 3. The compound was homogeneous on TLC (Rf 0.20, solvent system B) and could be recrystallized from EtOAc, m.p. : 153-155°C; $[\alpha]_D^{22}$: -11.8° (c=0.7; $CHCl_3$); ^{1}H-NMR : δ 7.61-7.07 (m, 5H, C_6H_5), 5.55 and 5.37 (2xs, 1H, CHCl), 4.69-4.42 and 4.42-4.19 (2xm, 1H $CHCO_2$), 3.77 and 3.67 (2xs, 3H, CO_2CH_3), 3.77-3.22 (m, 2H, CH_2N), 2.47-1.71 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); the ratio

of rotamers is approximately 8:1; exact mass calcd. for $C_{14}H_{17}ClNO_3$ (M^++1), m/e 282.0897, found : 282.0898; chemical-ionization mass spectrum, m/e (relative intensity) : 284/282 ($[M+1]^+$, 74%), 252/250 ($[M-OCH_3]^+$, 6%), 248 ($[M+2H-Cl]^+$, 35%), 246 ($[M-Cl]^+$, 50%), 224/222 ($[M-CO_2CH_3]^+$, 34%), 218 ($[M-Cl-CO]^+$, 100%), 188 ($[M+1-Cl-CO_2CH_3]^+$, 12%), 156 (12%), 130 ($[M+1-C_6H_5C(Cl)CO]^+$, 16%), 128 ($[M-C_6H_5CH(Cl)CO]^+$, 90%), 70 ($[C_4H_8N]^+$, 50%); anal. calcd. for $C_{14}H_{16}ClNO_3$: C, 59.68; H, 5.72; N, 4.97, found : C, 59.02; H, 5.67; N, 4.89.

N-[(R)-2-Chloro-2-phenyl-acetyl]-(S)-proline methyl ester 21b [57] was obtained in 28% yield from 3. The compound was homogeneous on TLC (Rf 0.24, solvent system B) and was recrystallized from EtOAc, m.p. : 107-109°C; $[\alpha]_D^{22}$: -105.8° (c=0.7; $CHCl_3$); ^{1}H-NMR : δ 7.63-7.13 (m, 5H, C_6H_5), 5.55 and 5.37 (2xs, 1H, CHCl), 4.76-4.29 (m, 1H, $CHCO_2$), 3.84-3.18 (m, 2H, CH_2N), 3.74 and 3.54 (2xs, 3H, CO_2CH_3), 2.43-1.70 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2N$); the ratio of rotamers is approximately 5:1; exact mass calcd. for $C_{14}H_{17}ClNO_3$ (M^++1), m/e 282.0897, found : 282.0895; chemical-ionization mass spectrum, m/e (relative intensity) : 284/282 ($[M+1]^+$, 97%), 252/250 ($[M-OCH_3]^+$, 8%), 248 ($[M+2H-Cl]^+$, 73%), 246 ($[M-Cl]^+$, 61%), 224/222 ($[M-CO_2CH_3]^+$, 50%), 218 ($[M-Cl-CO]^+$, 100%), 188 ($[M+1-Cl-CO_2CH_3]^+$, 33%), 156 (12%), 130 ($[M+1-C_6H_5C(Cl)CO]^+$, 35%), 128 ($[M-C_6H_5CH(Cl)CO]^+$, 78%), 70 ($[C_4H_8N]^+$, 39%); anal. calcd. for $C_{14}H_{16}ClNO_3$: C, 59.68; H, 5.72; N, 4.97, found : C, 59.20; H, 5.75; N, 4.90.

For the determination of the coalescence temperature (± 75°C for both diastereomers) CD_3NO_2 was used as solvent; in this solvent the ratio of rotamers was found to be approximately 8.5:1 for both diastereomers 21a and 21b.

General procedure for the synthesis of the bicyclic N-hydroxy-2,5-dioxo-piperazines 14-17.

To a solution of the relevant N-(2-halo-acyl)-(S)-proline methyl ester 10-13 or 21 (10 mmol) in 100 ml of dry MeOH $HONH_2$.HCl (6.95 g, 100 mmol) and t-BuOK (11.22 g, 100 mmol) were added successively. The resulting suspension was heated under reflux until completion of the reaction (2-72 hours) as monitored by TLC (solvent system B). After filtration of the reaction mixture the precipitate was washed thoroughly with MeOH/CH_2Cl_2 (10:90, v/v), and the filtrate was concentrated to dryness in vacuo. The solid residue was suspended in 150 ml of MeOH/CH_2Cl_2 (10:90, v/v) and stirred at room temperature for a while. Filtration followed by removal of the solvent under reduced pressure afforded the crude product, which was purified by column chromatography (solvent system D (14), F (15 and 16) or G (17a and b)).

The compounds thus obtained were contaminated slightly with Fe^{3+}, as could be deduced from their red colour. The impurity could be removed by treatment of a solution of the product with Spheron Oxine 1000 (0.025-0.040 mm),[87] a powder consisting of polymer-bound 8-hydroxyquinoline.[88] After filtration of the complexing agent evaporation of the solvent in vacuo furnished mostly pure product. Sometimes removal of residual traces of ferric ions had to be accomplished by washing the solid compound with a small amount of EtOAc.

Cyclo-[(S)-prolyl-N-hydroxyglycyl] 14 was obtained in 25% yield after recrystallization from CH_2Cl_2/CCl_4, m.p. : 142-144°C. The compound was homogeneous on TLC (Rf 0.32, solvent system E); ^{1}H-NMR: δ 6.63-5.49 (br, 1H, NOH), 4.63-4.01 (AB part of ABX spectrum and

m, 3H, CH_2NO and CHCO), 3.91-3.32 (m, 2H, CH_2N), 2.59-2.24 and 2.24-1.67 (2xm, 1H resp. 3H, $C\underline{H}_2C\underline{H}_2CH_2N$); IR (KBr) : 3360 (br), 2800 (br) and 1660 (br) cm^{-1}; exact mass calcd. for $C_7H_{10}N_2O_3$ (M^+), m/e 170.0691, found : 170.0687; electron-impact mass spectrum, m/e (relative intensity) : 170 (M^+, 20%), 154 ($[M-O]^+$, 17%), 153 ($[M-OH]^+$, 44%), 142 ($[M-CO]^+$, 20%), 125 ($[M-CH_2NOH]^+$, 17%), 112 (12%), 111 ($[M-CONOH]^+$, 21%), 98 (8%), 97 ($[M-C(O)CH_2NOH]^+$, 15%), 83 (24%), 70 ($[C_4H_8N]^+$, 100%), 69 (50%), 68 (20%), 56 (20%), 42 (19%), 41 (37%), 28 (21%).

Cyclo-[(S)-prolyl-(S)-N-hydroxy-alanyl] *15b* was obtained in 46% yield.[58] The compound was homogeneous on TLC (Rf 0.44, solvent system E) and could be recrystallized from CH_2Cl_2/CCl_4, m.p. : 151-153°C; $[\alpha]_D^{22}$: -161.2° (c=0.235; MeOH); ^{1}H-NMR : δ 4.56-4.25 (8 lines, 1H, CHNO), 4.25-3.96 (m, 1H, CHCO), 3.96-3.30 (m, 2H, CH_2N), 2.68-2.22 and 2.22-1.77 (2xm, 1H resp. 3H, $C\underline{H}_2C\underline{H}_2CH_2N$), 1.66 (d, 3H, CH_3); exact mass calcd. for $C_8H_{12}N_2O_3$ (M^+), m/e 184.0848, found : 184.0848; electron-impact mass spectrum, m/e (relative intensity) : 184 (M^+, 11%), 168 ($[M-O]^+$, 59%), 167 ($[M-OH]^+$, 17%), 166 ($[M-H_2O]^+$, 15%), 140 ($[M-CH_2NO]^+$, 11%), 125 ($[M-CH(CH_3)NOH]^+$, 34%), 123 (11%), 112 (6%), 98 (6%), 97 ($[M-C(O)CH(CH_3)NOH]^+$, 35%), 96 (6%), 70 ($[C_4H_8N]^+$, 100%), 69 (50%), 68 (23%), 55 (10%), 44 (46%), 43 (13%), 42 (26%), 41 (44%), 28 (32%).

Moreover, a mixture enriched in the other diastereomer 15a (ratio 4.5:1) was obtained in 6% yield. This mixture of diastereomers was also homogeneous on TLC (Rf 0.44, solvent system E); ^{1}H-NMR : δ 4.55-3.95 (m, 2H, CHNO and CHCO), 3.95-3.32 (m, 2H, CH_2N), 2.67-2.25 and 2.25-1.73 (2xm, 1H resp. 3H, $C\underline{H}_2C\underline{H}_2CH_2N$), 1.67 and 1.57 (2xd, together 3 lines, 3H, CH_3).

Cyclo-[(S)-prolyl-(R)-N-hydroxyvalyl] ***16a*** was obtained in 61% yield from 12a. The compound was homogeneous on TLC (Rf 0.49, solvent system E) and could be recrystallized from CH_2Cl_2/CCl_4, m.p. : 170-172°C, $[\alpha]_D^{22}$ = -145.5° (c=0.235; MeOH); ^{1}H-NMR : 4.22 and 4.22-4.02 (d resp. m, 2H, CHNO and CHCO), 3.88-3.30 (m, 2H, CH_2N), 2.73-2.24 and 2.24-1.61 (2xm, 2H resp. 3H, $C\underline{H}_2C\underline{H}_2CH_2N$ and $C\underline{H}(CH_3)_2$), 1.10 and 1.07 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{10}H_{16}N_2O_3$ (M^+), m/e 212.1161, found : 212.1165; electron-impact mass spectrum, m/e (relative intensity) : 212 (M^+, 9%), 195 ($[M-OH]^+$, 9%), 170 ($[M-CH_3C(H)CH_2]^+$, 25%), 153 ($[M-CONOH]^+$, 45%), 141 ($[M-C(O)CHNO]^+$, 54%), 125 ($[M-CH(i-C_3H_7)NOH]^+$, 5%), 98 ($[M-C(O)C(i-C_3H_7)NOH$, 5%), 70 ($[C_4H_8N]^+$, 100%), 55 (7%), 41 (24%), 28 (10%); anal. calcd. for $C_{10}H_{16}N_2O_3$: C, 56.59; H, 7.60; N, 13.20, found : C, 56.18; H, 7.56; N, 13.07.

The mixture of 16a and 16a', obtained in 20% yield by ring closure of 12b, showed exactly the same data with the exception of the specific rotation; $[\alpha]_D^{22}$: +81.2° (c=0.235; MeOH).

Cyclo-[(S)-prolyl-(R)-N-hydroxyphenylglycyl] ***17a*** was obtained in 75% yield from 13a. The compound was homogeneous on TLC (Rf 0.49, solvent system E) and was recrystallized from $MeOH/CH_2Cl_2$, m.p. : 207-209°C(d); $[\alpha]_D^{20}$: -111.7° (c=0.298; MeOH); ^{1}H-NMR: δ 7.37 (s, 5H, C_6H_5), 5.41 (s, 1H, CHNO), 4.31-4.01 (m, 1H, CHCO), 3.87-3.30 (m, 2H, CH_2N), 2.64-2.29 and 2.29-1.70 (2xm, 1H resp. 3H, $C\underline{H}_2C\underline{H}_2CH_2N$); IR (KBr) : 3070, 2880, 1670 and 1630 cm^{-1}; exact mass calcd. for $C_{13}H_{14}N_2O_3$ (M^+), m/e 246.1004, found : 246.1003; electron-impact mass spectrum, m/e (relative intensity): 246 ($[M^+]$, 21%), 229 ($[M-OH]^+$, 37%), 201 ($[M-CH_2NOH]^+$, 14%), 132 ($[C_6H_5CH=N-C=O]^+$, 100%), 121 ($[C_6H_5-CH=N-OH]^+$, 16%), 77 (23%), 70

($[C_4H_8N]^+$, 40%), 51 (11%), 41 (14%), 28 (29%); anal. calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38, found: C, 63.50; H, 5.73; N, 11.37.

Ring closure of 21b afforded a mixture of 17a and 17b in 54% yield after column chromatography (solvent system G). After a second elution over silica gel (solvent system G) *cyclo-[(S)-prolyl-(S)-N-hydroxyphenylglycyl] 17b* was isolated in 14% yield, based on 21b. The compound was homogeneous TLC (Rf 0.46, solvent system E) and was recrystallized from CH_2Cl_2/CCl_4, m.p. : 184-186°C; $[\alpha]_D^{20}$: -47.8° (c= 0.45; MeOH); ^{1}H-NMR: δ 7.51 - 7.11 (m, 5H, C_6H_5), 5.32 (d, 1H, CHNO), 4.40 - 4.11 (m, 1H, CHCO), 4.02 - 3.29 (m, 2H, CH_2N), 2.69-2.39 and 2.28-1.74 (2xm, 1H resp. 3H, $CH_2CH_2CH_2N$); IR (KBr) : 3110 (br), 2890 (br), 1660 and 1625 cm^{-1}; exact mass calcd. for $C_{13}H_{14}N_2O_3$ (M^+), m/e 246.1004, found: 246.1007; electron-impact mass spectrum, m/e (relative intensity): 246(M^+, 36%), 229 ($[M-OH]^+$, 34%), 201 ($[M-CH_2NOH]^+$, 12%), 132 ($[C_6H_5CH=N-C=O]^+$, 100%), 121 ($[C_6H_5-CH=N-OH]^+$, 23%), 77 (28%) 70 ($[C_4H_8N]^+$, 45%), 51 (12%), 41 (18%), 28 (7%); anal. calcd. for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38, found : C, 62.93; H, 5.76; N, 11.17.

2-Benzyloximino-3-phenyl-propanoic acid 23

Phenylpyruvic acid sodium salt monohydrate 22 (7.71 g, 37.8 mmol) was suspended in 160 ml of $CHCl_3$. After the addition of O-benzylhydroxylamine hydrochloride salt (6.03 g, 37.8 mmol) the suspension was stirred at room temperature for 24 hours. Then 160 ml of H_2O was added and stirring was continued until complete dissolution of all solids. The two clear layers were separated after which the organic layer was dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded 23 as a white solid in 95% yield.

Compound 23 was used in the next reaction without further purification; ^{1}H-NMR : δ 7.31 and 7.22 (2xs, 10 H, 2x C_6H_5), 5.29 (s, 2H, CH_2ON), 3.91 (s, 2H, CH_2CN); IR (CCl_4) : 3600-2500, 1770 and 1700 cm^{-1}.

2-Benzyloximino-3-phenyl-propanoyl chloride 24

A solution of $SOCl_2$ (12.6 g, 106 mmol) in 28 ml of dry toluene was added at room temperature to a stirred suspension of 23 (9.30 g, 34.5 mmol) in 35 ml of dry toluene. Stirring was continued for 2h at 80°C after which the solvent and excess $SOCl_2$ were evaporated in vacuo. To remove residual $SOCl_2$ and liberated SO_2, 70 ml of dry toluene was added and evaporated in vacuo; this was repeated twice. Finally residual traces of toluene were removed at a high vacuum pump. The crude product was obtained in 97% yield; ^{1}H-NMR : δ 7.35 and 7.17 (2xs, 10H, 2x C_6H_5), 5.38 (s,2H, CH_2ON), 3.87 (s, 2H, CH_2CN); IR (CCl_4) : 1740 cm^{-1}. The compound was used immediately in the next reaction without further purification.

N-(2-Benzyloximino-3-phenyl-propanoyl)-(S)-proline methyl ester 25

A solution of triethylamine (3.03 g, 30 mmol) in 5 ml of dry CH_2Cl_2 was added dropwise to a stirred and chilled (0°C) solution of 3 (2.49 g, 15 mmol) and crude 24 (4.32 g, 15 mmol) in 20 ml of dry CH_2Cl_2. Stirring was continued at room temperature for 16 hours. Then water was added to dissolve all solids. The resulting clear layers were separated, whereupon the organic layer was dried over Na_2SO_4. The residue, obtained after evaporation of the solvent in vacuo, was purified by column chromatography (solvent system B), thus affording 25 as a yellow oil in 78% yield. The compound was homogeneous on TLC (Rf 0.40, solvent system B); ^{1}H-NMR: δ 7.28 and

7.21 (2xs, 10H, 2x C_6H_5), 5.18 and 5.13 (2xs, 2H, CH_2ON), 4.80-4.62 and 4.55-4.35 (2x m, 1H, $CHCO_2$), 4.05 and 3.91 (AB-spectrum, J_{AB} = 13.2 Hz) and 3.97 (s, together 2H, CH_2CN), 3.77-3.31 (m, 2H, CH_2N), 3.68 and 3.49 (2xs, 3H, CO_2CH_3), 2.31-1.52 (m, 4H, $C\underline{H_2}C\underline{H_2}CH_2N$); according to this spectrum equal amounts of two rotamers are present. For the determination of the coalescence temperature (70-75°C) CD_3NO_2 was used as solvent; in this solvent the ratio of rotamers was found to be approximately 1.5:1; exact mass calcd. for $C_{22}H_{25}N_2O_4$ (M^++1), m/e 381.1814, found: 381.1810; chemical-ionization mass spectrum, m/e (relative intensity): 471 ($[M+C_7H_7]^+$, 10%), 381 ($[M + 1]^+$, 100%), 321 ($[M-CO_2CH_3]^+$, 8%), 273 ($[M-C_7H_7O]^+$, 11%), 128 ($[M-C_6H_5CH_2-C(=N-OBzl)-C(O)]^+$, 84%), 118 ($[C_8H_8N]^+$, 16%), 107 ($[C_7H_7O]^+$, 16%), 91 ($[C_7H_7]^+$, 75%), 79 (16%), 70 ($[C_4H_8N]^+$, 20%).

N-Benzyloxy-(R,S)-phenylalanyl-(S)-proline methyl ester 26

Trimethylamine-borane (0.38 g, 5.2 mmol) was added at regular intervals to a stirred solution of 25 (1.01 g, 2.6 mmol) in 10 ml of dry dioxane, saturated with dry HCl. While stirring was continued at room temperature altogether 4.36 g (23 eq.) of the reducing agent was added spread over 10 days. Although the conversion was not complete, as monitored by TLC, the reaction was interrupted after 11 days. The reaction mixture was concentrated in vacuo. The resulting jelly-like material was dissolved in CH_2Cl_2 and washed with 5% aqueous $NaHCO_3$ (w/v) and brine. The organic layer was dried over Na_2SO_4 and then the solvent was evaporated in vacuo. Prior to purification of the residue by column chromatography (solvent system B), part of the borates was removed by crystallization from CH_2Cl_2/n-hexane. In addition to a

small amount of starting material (7%), pure 26 was obtained as an oil in 26% yield. The compound was homogeneous on TLC (Rf 0.17, solvent system B); ^{1}H-NMR : δ 7.45-7.08 (m, 10H, 2x C_6H_5), 4.77-4.45 (m, 1H, $CHCO_2$), 4.61 (s, 2H, $NOCH_2$), 3.87-2.85 (m, 3H, CH_2N and CHNO), 3.71 (s, 3H, CO_2CH_3), 2.75 (d, 2H, CH_2CHNO), 2.39-1.51 (m, 4H, $CH_2CH_2CH_2N$); exact mass calcd. for $C_{22}H_{27}N_2O_4$ (M^++1), m/e 383.1971, found : 383.1968; chemical-ionization mass spectrum, m/e (relative intensity) : 383 ($[M+1]^+$, 100%), 351 ($[M-OCH_3]^+$, 3%), 291 ($[M-C_7H_7]^+$, 32%), 275 ($[M-C_7H_7O]^+$, 5%), 254 ($[C_6H_5CH_2CH(NHOBzl)CO]^+$, 8%), 245 ($[M+1-OCH_3-C_7H_7O]^+$, 17%), 226 ($[C_6H_5CH_2CH(NHOBzl)]^+$, 29%), 130 (49%), 128 ($[M-C_6H_5CH_2CH(NHOBzl)CO]^+$, 20%), 107 ($[C_7H_7O]^+$, 16%), 91 ($[C_7H_7]^+$, 61%), 79 (14%), 70 ($[C_4H_8N]^+$, 28%).

Methyl E-α-phenylcinnamate 33

A solution of α-phenylcinnamic acid 32 (*cis*-form[89], 11.2 g, 50 mmol) was added dropwise to a stirred solution of an excess of diazomethane (100 mmol) in 400 ml of dry ether.[90] During this procedure the reaction mixture was kept at 0°C. After completion of the addition the reaction mixture was stirred at room temperature overnight. The excess of diazomethane was removed by adding a few drops of acetic acid, after which the solvent was evaporated in vacuo. The product, obtained in quantitative yield as a yellow oil which solidified quickly, was used in the next reaction without further purification; ^{1}H-NMR : δ 7.84 (s, 1H, C_6H_5CH), 7.59-6.81 (m, 10H, 2x C_6H_5), 3.77 (s, 3H, CO_2CH_3); exact mass calcd. for $C_{16}H_{14}O_2$ (M^+), m/e 238.0994, found: 238.0986; electron-impact mass spectrum, m/e (relative intensity): 238 (M^+, 78%), 180 (50%), 179 ($[M-CO_2CH_3]^+$, 100%), 178 (76%), 165 (15%), 152 (12%), 121 (88%), 89 (17%), 77(13%), 76 (13%), 51 (14%).

(E)-α-Phenylcinnamyl alcohol 31 ((E)-2,3-diphenyl-2-propenol)

A 1.0M solution (22 ml, 22 mmol) of DIBAL-H in n-hexane was added carefully to a chilled (0°C) solution of 33 (2.38 g, 10 mmol) in 80 ml of dry toluene under an argon atmosphere. After stirring at 0°C for 30 min. and at room temperature for 1 hour the reaction was complete as judged by TLC (solvent system H). The reaction mixture was quenched with 2.5% (w/v) aqueous NH_4Cl (100 ml), whereupon the resulting pulp was filtered by suction using a sintered glass funnel. After adding EtOAc (100 ml) to the filtrate, the two layers were separated. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (solvent system H), affording pure 31 in 66% yield. The product was homogeneous on TLC (Rf 0.27, solvent system H); m.p. : 70-72°C (lit.[91] : 71-73°C); ^{1}H-NMR: δ 7.34-6.85 (m, 10H, 2xC_6H_5), 6.62 (s, 1H, $C_6H_5C\underline{H}$), 4.40 (br.s., 2H, CH_2O), 1.72 (br., 1H, OH); (d^6-DMSO): δ 7.41- 6.81 (m, 10H, 2x C_6H_5), 6.60 (s, 1H, $C_6H_5C\underline{H}$), 5.17 (br., 1H, OH), 4.19 (br.s., 2H, CH_2O); UV : λ_{max} (EtOH), 222 and 255 nm (lit.[91] : 222 and 257 nm); exact mass calcd. for $C_{15}H_{14}O$ (M^+), m/e 210.1045, found : 210.1042; chemical-ionization mass spectrum, m/e (relative intensity): 211 ($[M+1]^+$, 3%), 210 (M^+, 13%), 194 (16%), 193 ($[M-OH]^+$, 100%), 139 (25%), 91 ($[C_7H_7]^+$, 11%), 49 (11%).

Besides, a small amount (< 1%) of the Z-isomer was isolated; Rf 0.30 (solvent system H); ^{1}H-NMR : δ 7.65-7.13 (m, 10H, 2x C_6H_5), 6.93 (s, 1H, $C_6H_5C\underline{H}$), 4.69 (s, 2H, CH_2O), 1.55 (s, 1H, OH); UV : λ_{max} (EtOH), 271 nm (lit.[91] : 273 nm); exact mass calcd. for $C_{15}H_{14}O$ (M^+), m/e 210.1045, found : 210.1050; chemical-ionization mass spectrum, m/e (relative intensity) : 211 ($[M+1]^+$, 4%), 210

(M^+, 18%), 194 (19%), 193 ($[M-OH]^+$, 100%), 105 (10%), 91 ($[C_7H_7]^+$, 10%).

General procedure for the epoxidation of allylic alcohols in the presence of one of the hydroxamic acids 14-17

The allylic alcohol (3 mmol), the catalyst (V, Ti or Mo: 1-10 mol%) and the hydroxamic acid (1.1 - 21 mol%) were dissolved successively in 60 ml of dry CH_2Cl_2 under an argon atmosphere. The resulting solution was stirred at room temperature for 30 minutes. After adjustment of the desired reaction temperature (*i.e.* 0°C or 20°C), 1.5 ml of a 4.0M solution of tert-butyl hydroperoxide (6 mmol) in dichloroethane or dichloromethane was added dropwise.[92] Stirring was continued at room temperature[93] until completion of the reaction (1-10 days) as monitored by TLC (solvent system B).[94] Then the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography (solvent system I for 34, B for 35, and H for 36), affording the relevant epoxy alcohol in 17-89% yield.

trans-3-(n-Butyl)-oxiranemethanol 34 : Rf 0.20 (solvent system I); ^{1}H-NMR : δ 3.95-3.45 (8 lines, 2H, CH_2O), 3.08 (br., 1H, OH), 3.01-2.84 (m, 2H, CHOCH), 1.80-1.10 (m, 6H, $CH_2CH_2CH_2$), 0.89 (m, 3H, CH_3); exact mass calcd. for $C_7H_{15}O_2$ (M^+ + 1), m/e 131.1072, found : 131.1070; chemical-ionization mass spectrum, m/e (relative intensity) : 131 ($[M + 1]^+$, 15%) 113 ($[M-OH]^+$, 29%), 95 (100%), 69 (36%), 57 ($[C_4H_9]^+$, 8%), 43 (10%).

Epoxygeraniol 35 : Rf 0.17 (solvent system I); ^{1}H-NMR : δ 5.09 (br.t., 1H, $C\underline{H}C(CH_3)_2$), 4.00-3.52 (m, 2H, CH_2O), 3.00 (dd, 1H, $C\underline{H}CH_2O$), 2.36-1.34 (m, 5H, CH_2CH_2 and OH), 1.71 and 1.63 (2xs, 6H,

$C(CH_3)_2$), 1.33 (s, 3H, CH_3); exact mass calcd. for $C_{10}H_{19}O_2$ (M^++1), m/e 171.1385, found : 171.1385; chemical-ionization mass spectrum, m/e (relative intensity): 171 ($[M+1]^+$, 4%), 153 ($[M-OH]^+$, 30%), 135 (49%), 123 (22%), 109 (70%), 95 (41%), 83 (32%), 69 (100%), 55 (18%), 41 (44%).

trans-2,3-Diphenyloxiranemethanol 36 : Rf 0.49 (solvent system B); ^{1}H-NMR: δ 7.17 (s, 5H, C_6H_5), 7.17-6.95 (m, 5H, C_6H_5), 4.50 (s, 1H, $C_6H_5C\underline{H}$), 4.04 (d, 2H, CH_2O), 1.95 (t, 1H, OH); exact mass calcd. for $C_{15}H_{15}O_2$ (M^++1), m/e 227.1072, found : 227.1067; chemical-ionization mass spectrum, m/e (relative intensity): 227 ($[M+1]^+$, 30%), 209 ($[M-OH]^+$, 69%), 181 ([stilbene + 1]$^+$, 46%), 167 (58%), 131 (23%), 120 (33%), 105 (100%), 91 (22%).

General procedure for the determination of the enantiomeric excess of the epoxy alcohols.

Pyridine (0.166 g, 2.1 mmol) was added dropwise to a chilled (0°C) solution of the epoxy alcohol (2 mmol) and freshly distilled acetyl chloride (0.165 g, 2.1 mmol) in 6 ml of dry CH_2Cl_2.[95] Subsequently the reaction mixture was stirred at room temperature until completion of the reaction as monitored by TLC (solvent system B). The reaction mixture was washed successively with 0.1N aqueous HCl, H_2O, 1% (w/v) aqueous $NaHCO_3$ and H_2O. After drying over Na_2SO_4, the organic layer was concentrated in vacuo. The residue was purified by column chromatography (solvent system B), affording the epoxy acetates in approximately 85% yield. The e.e. of these compounds was established by ^{1}H-NMR spectroscopy using the chiral shift reagent tris-(3-(heptafluoro-propylhydroxymethylene)-d-camphorato) europium(III)($Eu(hfc)_3$, Janssen Chimica).

trans-3-(n-Butyl)-oxiranemethyl acetate (from 34); ^{1}H-NMR : δ 4.43-3.79 (AB part of ABX spectrum, 2H, CH_2O), 3.03-2.75 (m, 2H, CHOCH), 2.08 (s, 3H, $CH_3C(O)$), 1.67-1.17 (m, 6H, $CH_2CH_2CH_2$), 0.90 (m, 3H, CH_3); exact mass calcd. for $C_9H_{17}O_3$ (M^++1), m/e 173.1178, found : 173.1172; chemical-ionization mass spectrum, m/e (relative intensity): 173 ($[M+1]^+$, 61%), 155 ($[M-OH]^+$, 17%), 131 (6%), 113 ($[M-CH_3CO_2]^+$, 31%), 95 (100%), 69 (21%), 57 (7%), 43 (91%).

Epoxygeranyl acetate (from 35) : Rf 0.45 (solvent system I); ^{1}H-NMR: δ 5.05 (br.t., 1H, C<u>H</u>C$(CH_3)_2$), 4.38-3.88 (AB part of ABX spectrum, 2H, CH_2O), 2.96 (X part of ABX spectrum (dd), 1H, C<u>H</u>CH_2O), 2.24-1.35 (m, 4H, CH_2CH_2), 2.08 (s, 3H, $CH_3C(O)$), 1.66 and 1.60 (2xs, 6H, $C(CH_3)_2$), 1.29 (s, 3H, CH_3); exact mass calcd. for $C_{12}H_{21}O_3$ (M^++1), m/e 213.1491, found : 213.1484; chemical-ionization mass spectrum, m/e (relative intensity): 213 ($[M+1]^+$, 18%), 195 ($[M-OH]^+$, 5%), 153 ($[M-CH_3CO_2]^+$, 40%), 135 (97%), 127 (20%), 109 (40%), 95 (22%), 69 (60%), 43 (55%).

trans-2,3-Diphenyloxiranemethyl acetate (from 36); ^{1}H-NMR : δ 7.15 (s, 5H, C_6H_5), 7.15-6.88 (m, 5H, C_6H_5), 4.61 and 4.35 (AB spectrum, J_{AB} = 12.5 Hz, CH_2O), 4.30 (s, 1H, C_6H_5C<u>H</u>), 2.06 (s, 3H, CH_3).

Asymmetric oxidation of thioanisole 38

Vanadyl acetylacetonate (7.9 mg, 0.03 mmol), hydroxamic acid 17a or 17b (22-37 mg, 0.09-0.15 mmol) and thioanisole 38 (0.372 g, 3 mmol) were dissolved successively in 60 ml of dry CH_2Cl_2 under an argon atmosphere. After stirring at room temperature for 15 minutes the solution was cooled to 0°C. Then 1.5 ml of a 4.0M solution of TBHP[92] (6 mmol) was added dropwise, whereupon the reaction mixture was

stored in an refrigerator. The reaction was interrupted after 9-18 days, depending on the amount of hydroxamic acid used, although the conversion was not complete as judged by TLC (solvent system H or J). Water (20 ml) was added dropwise to the chilled (0°C) reaction mixture. The layers were separated after stirring at 0°C for 15 minutes and at room temperature for 30 minutes. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography (solvent system K), affording pure methyl phenyl sulfoxide 39; ^{1}H-NMR (60 MHz): δ 7.70-7.25 (m, 5H, C_6H_5), 2.66 (s, 3H, CH_3).

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55. This compound was obtained by repeated treatment of 4 with

CD_3OD/D_2O, followed by removal of the solvent under high vacuum. The product was stored in a vacuum desiccator over phosphorous pentoxide.

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57. The tentative assignment of the absolute configurations was based on the observation that 13a and 21a yielded the same product on treatment with H_2NOH.
58. The initial fractions afforded 15b. In the ^1H-NMR spectra of both diastereomers a five-bond longe range coupling between the α-carbon atom protons, *i.e.* C_3-H and C_6-H, could be observed. Since it has been reported that for cyclic dipeptides containing proline $^5J(HH)$ is greater in the *cis*-isomer than in the *trans*-isomer, structure 15b was assigned to the diastereomer showing the greater $^5J(HH)$. See for comparison : Davies, D.B.; Khaled, M.A., J. Chem. Soc. Perkin II (1976), 187 and references cited therein.
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64. We are indebted to Mr. L. Thys (Department of Organic Chemistry, University of Nijmegen) for supplying us with an amount of this compound.
65. The e.e. was determined by ^1H-NMR spectroscopy, using the chiral shift reagent $Eu(hfc)_3$, after conversion of the epoxy alcohol into the corresponding acetate (see experimental section).
66. As far as epoxygeraniol 35 is concerned, the absolute configuration of the enantiomer formed in excess is indicated in parentheses.
67. The specific rotation of this product was $[\alpha]^{20}(D)$: -17.6° (c=0.96; $CHCl_3$); the specific rotation of material showing an e.e. of at least 95% was found to be : $[\alpha]^{20}(D) = \pm 45.1°$ (c=0.96; $CHCl_3$). (Mr. L. Thys, Department of Organic Chemistry, University of Nijmegen, personal communication).
68. The specific rotation of this product was $[\alpha]^{20}(D)$: -2.7° (c=1.5; $CHCl_3$); the specific rotation of (2S,3S)-epoxygeraniol, having an e.e. of at least 94%, was shown to be $[\alpha]^{24}(D)$: -6.36° (c=1.5; $CHCl_3$) (see ref. 60).
69. In this case toluene instead of CH_2Cl_2 was used as solvent.

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74. It must be emphasized that the absolute configurations of these compounds were assigned tentatively (see also sections 3.2.2 and 3.2.4).
75. a) These reactions proceeded faster than the corresponding conversions involving 17a as chiral ligand; b) In this experiment an opposite chiral induction is observed in comparison with all other experiments involving 31 as substrate.
76. It has been demonstrated that $Mo(CO)_6$ is oxidized by TBHP to a catalytically active Mo^{6+}-species, see e.g. : Sheldon, R.A., Recl. Trav. Chim. Pays-Bas (1973), *92*, 253, 367.
77. These conversions were rather slow and interrupted before completion; some starting material could be recovered from the reaction mixture. As a result of the presence of too many equivalents (3) of hydroxamic acid much $MoO_2(17a)_2$ may be formed in the Mo^{6+}-catalyzed reaction. In the bishydroxamate species probably not enough coordination sites are available for the substrate and TBHP. If no hydroxamic acid was added, the Mo^{6+}-catalyzed reaction proceeded smoothly. On the other hand, even in the absence of hydroxamic acid, we did not succeed in achieving complete conversions in the Ti^{4+}-catalyzed epoxidations. Later on Sharpless reported (ref. 61, 63) that in such cases complete conversions can be realized only by adding molecular sieves to the reaction mixture.
78. For a few examples of such complexes, see : a) Dutta, R.L.; Lahiry, S., J. Indian Chem. Soc. (1962), *39*, 860; b) Dutta, R.L.; Lahiry, S., J. Indian Chem. Soc. (1963), *40*, 53; c) Dutta, R.L.; Chatterjee, B., J. Indian Chem. Soc. (1967), *44*, 780; d) Bag, S.P.; Khastagir, A.K., J. Indian Chem. Soc. (1977), *54*, 254; e) Tomioka, H.; Takai, K.; Oshima, K.; Nozaki, H., Tetrahedron Lett. (1980), *21*, 4843; f) Williams, I.D.; Pedersen, S.F.; Sharpless, K.B.; Lippard, S.J., J. Am. Chem. Soc. (1984), *106*, 6430, and also ref. 34 and 35.
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Kagan, H.B., J. Am. Chem. Soc. (1984), *106*, 8188.
82. In parentheses the absolute configuration of the enantiomer formed in excess is indicated; see for comparison ref. 81.
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87. We are grateful to Dr. J.D.M. Herscheid (Radionuclide Centre (RNC), Free University, Amsterdam) for supplying us with an amount of this material.
88. a) For more information on the complexation of metal-ions by 8-hydroxyquinoline, see e.g. : Phillips, J.P., Chem. Rev. (1956), *56*, 271; b) The removal of ferric ions from hydroxamic acid-iron complexes by treatment with an excess of the powerful chelating agent 8-hydroxyquinoline has been described before, see e.g. : Keller-Schierlein, W.; Diekmann, H., Helv. Chim. Act. (1970), *53*, 2035.
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93. In some cases the addition of TBHP was carried out at 0°C; subsequently the reaction mixture was stored in a refrigerator until completion of the reaction.
94. The presence of TBHP in the reaction mixture can be demonstrated by a starch-iodide test. In the case where the reaction was stopped before completion, and where there was still some TBHP present, the reaction mixture was quenched with trimethylphosphite.
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CHAPTER IV

SYNTHESIS OF N-HYDROXY ARNSTEIN TRIPEPTIDES : PROPOSED INTERMEDIATES IN THE BIOSYNTHESIS OF PENICILLIN

SYNTHESIS OF N-HYDROXY ARNSTEIN TRIPEPTIDES : PROPOSED INTERMEDIATES IN THE BIOSYNTHESIS OF PENICILLIN

4.1 *INTRODUCTION*

More or less by chance Alexander Fleming (1929)[1] observed antibiosis between a Penicillium mold and neighbouring bacterial cultures. The antibacterial substance of Fleming's experiments proved to be penicillin,[2] the oldest recognized antibiotic. The compound was the first microbial metabolite showing sufficient separation between toxicity to the bacterial cell and toxicity to the mammalian host to permit its use in the systematic treatment of bacterial infections of humans and animals.

The recognition of the potential value of penicillin in medicine led to the inauguration of a monumental Anglo-American research programme during World War II aimed at producing the antibiotic in sufficient quantities for widespread use.[3] The success of this enterprise was due largely to the rapid development of a fermentation process for large scale production of the antibiotic and to the selection of Penicillium strains superior for antibiotic synthesis.

Penicillin has turned out to be the progenitor of an important family of chemotherapeutic agents, the β-lactam antibiotics.[4,5] Even over 40 years after their introduction, these compounds are still the most widely prescribed antibiotics used in medicine. The β-lactam antibiotics are made available for use in human medicine by virtue of microbial synthesis. Although totally synthetic methods

have been developed for the production of β-lactam drugs,[3-10] currently all clinically used members of this class of antibiotics derive ultimately from a microbial source : fermentation still affords large quantities of β-lactam compounds for use as antibiotics or as intermediates for semisynthetic variants.[11]

Biogenesis of β-lactam antibiotics has intrigued scientists already for decades. The investigation of the biosynthesis of the β-lactam antibiotics is not only of fundamental scientific importance, but has also some utilitarian aspects. The acquired knowledge may be used to improve the yield of penicillin from fermentation methods and to induce the production of new penicillins by adding appropriate precursors to the cultures. Especially the latter objective is very important and needs to be explained more fully.

First and foremost is the constant need for new antibiotics with either different or broader antibacterial activities. A second spur is the search for β-lactam antibiotics to combat bacteria which have built up a resistance against the more traditional penicillins. To be superior, any new antibiotic must at least resemble penicillin in its mode of action -inhibition of bacterial cell wall synthesis- and in its freedom from serious toxic effects.

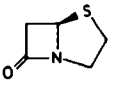
The first part of this chapter, *i.e.* 4.2 and 4.3, reviews our current knowledge of the biosynthesis of penicillin with particular emphasis on the youngest developments.

The second part, *i.e.* 4.4-4.8, describes our attempts to verify the postulate that an N-hydroxy tripeptide might be involved in penicillin biosynthesis.

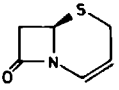
4.2 *STRUCTURE, OCCURRENCE AND BIOCHEMICAL MODE OF ACTION OF THE PENICILLINS AND CEPHALOSPORINS*

The discussion concerning the biosynthesis of penicillin will cover to a certain extent the biosynthesis of cephalosporins too, since there exists a close biogenetic relationship between these two classes of compounds.

The naturally occurring penicillins and cephalosporins are derivatives of a *cis*-fused β-lactam-thiazolidine ring system (penam) 1 and a *cis*-fused β-lactam-dihydrothiazine ring system (ceph-3-em) 2, respectively.



1, penam



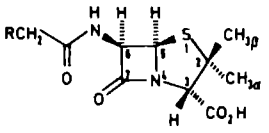
2, ceph-3-em

The substitution pattern of the ring systems depends largely upon the organism producing the compound and the fermentation conditions. Actually one can distinguish between two kinds of fermentation.[12-16]

One type, characterized by the potential production of an extensive range of penicillin antibiotics, has been classified as the Penicillium type. An example of an organism exhibiting this kind of fermentation is Penicillium chrysogenum. This mold incorporates many monosubstituted acetic acids (RCH_2CO_2H, where R is a nonpolar moiety) into penicillins so that the acid becomes the acyl substituent attached to the C-6 nitrogen in the penicillin 3 via an amide linkage.

Such aliphatic or aryl-substituted aliphatic carboxylic acids, or analogues which can easily generate such acids in vivo, can be present endogenously or added exogenously. Of the many penicillins biosynthetically producable this way, particularly benzylpenicillin

(penicillin G; 3, R= C_6H_5) and phenoxymethylpenicillin (penicillin



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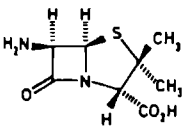

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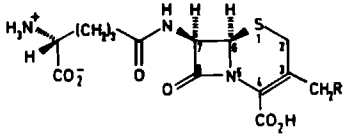
V; 3, R= C_6H_5O) are of great clinical utility. Two other products accumulate in this type of fermentation, especially when the availability of side-chain precursors is limited. These are isopenicillin N (3, R= HO_2C-$CH(NH_2)(CH_2)_2$), which has a 6-linked L-α-aminoadipyl side-chain, and 6-aminopenicillanic acid (6-APA; 4). The latter compound is an important intermediate in the preparation of the so-called semi-synthetic penicillins, produced by acylation of the free amino group of 4.

Cephalosporium acremonium typifies the organisms which show the other pattern of fermentation which is called the Cephalosporium type. These organisms excrete penicillin N, which has a 6-linked D-α-aminoadipyl side-chain, as the sole penicillin. It took some time before it was discovered that this compound is formed intracellularly by epimerization of the primary fermentation product isopenicillin N, having the L-configuration in the side-chain.[15,17,29] It is this type of fermentation which also yields a number of cephalosporin compounds, *e.g.* 5, R=H, OH or O-C(O)CH_3.



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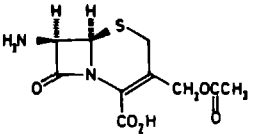

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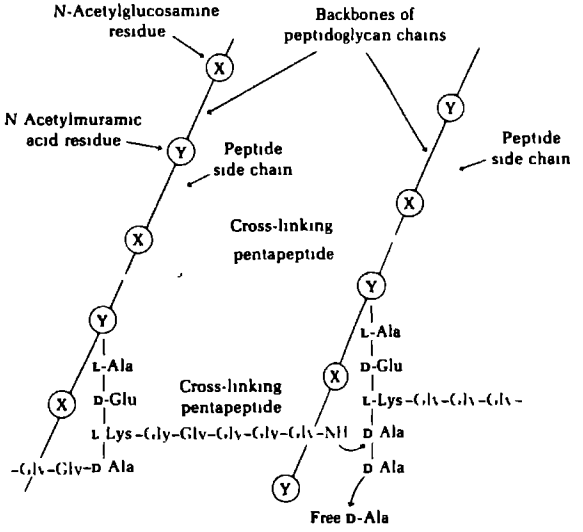
The Cephalosporium type of fermentation so far encountered differs from the Penicillium type in several aspects. It is insensitive to the addition of side-chain precursors to the fermentation medium. The cephalosporins and the penicillins which are excreted, have invariably the D-α-aminoadipyl side-chain. The amount of 6-APA (4) or 7-aminocephalosporanic acid (7-ACA; 6) which is formed in a Cephalosporium fermentation appears to be minimal or nil.

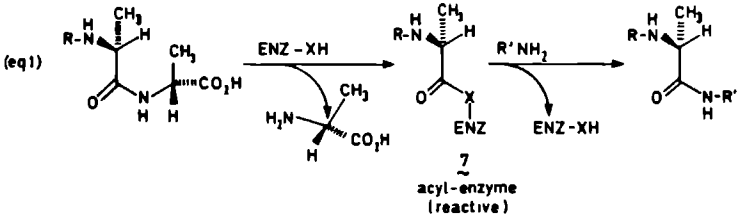
As was mentioned already in the introduction of this chapter, the curative effect of the β-lactam antibiotics is attributable to their ability to inhibit bacterial cell wall synthesis. The bacterial cell wall is a macromolecular network that surrounds the cell completely and provides its structural integrity. Of the several constituents of the cell wall, it is mainly the peptidoglycan that determines the cell shape and imparts the rigidity necessary to protect the bacterium from osmotic rupture.[18,19] Except for minor variations and modifications, all bacterial peptidoglycans are similar in that they are built up of long, linear polysaccharide chains of alternating N-acetylglucosamine- and N-acetylmuramyl peptide residues (see figure 1). These chains, extending in one direction, can be cross-linked directly or indirectly via the peptide side-chains. In the latter case the cross-link includes an short additional peptide. The length of such cross-linking peptides and their amino acid components vary with the species, *e.g.* in Staphylococcus aureus the chain is pentaglycine (figure 1). The extent of cross-linking varies with the bacterial species and growth conditions, and can range from as low as 25% in E. coli to more than 90% in S. aureus.

Fig. 1 : Completion of an indirect cross-link between two adjacent peptidoglycan chains in the bacterial cell wall



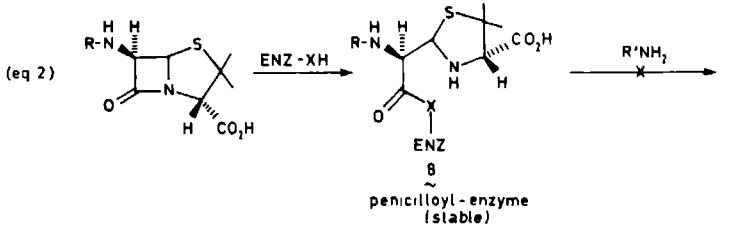
The cross-linkage between parallel peptidoglycan chains is realized in the last stage of the cell wall synthesis through a transpeptidation reaction in which the amino-terminal glycine residue of the cross-linking chain displaces the carboxyl-terminal D-alanine from the end of the pentapeptide side chain of an N-acetylmuramylpentapeptide residue in the adjacent peptidoglycan chain. It is this reaction that is inhibited by β-lactam antibiotics.[18-21]

The transpeptidase involved in this reaction reacts with its



substrate, an N-acetylmuramylpentapeptide residue, to form an acyl-enzyme intermediate 7 with release of the terminal D-alanine residue (eq.1). This acyl-enzyme intermediate 7 then reacts with an amino group (*e.g.* Gly-NH_2, fig. 1) from an adjacent peptidoglycan strand.

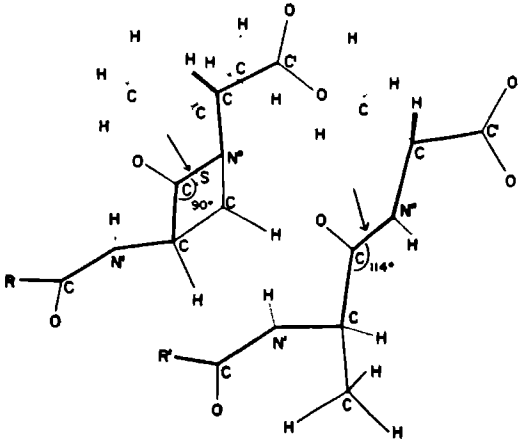
Nowadays it is generally accepted that penicillin inhibits the transpeptidase by forming a covalent bond with an amino acid residue (serine?[19,26]) at the active site of the enzyme (eq.2).[19-21]



Probably the resulting penicilloyl-enzyme 8 is relatively stable and resistant to attack by cell wall amino groups. Hence, the transpeptidase is irreversibly inhibited by penicillin.

It has been proposed that penicillin inactivates the peptidoglycan transpeptidase so effectively by acting as a structural analog of the enzyme's natural substrate, the acyl-D-alanyl-D-alanine terminus of the nascent peptidoglycan strand (see fig./eq.1). This theory is known as the "substrate analog hypothesis".[21,22] When molecular models of penicillin and acyl-D-alanyl-D-alanine are compared indeed a conformational similarity is observed (fig. 2). In contrast with normal amide bonds, the amide bond in the β-lactam ring is very reactive. X-ray analysis has shown that the N-atom in the β-lactam ring is distinctly pyramidal in both the penicillins and the cephalosporins[23], indicating a lower amide resonance stabilization than in normal amides.

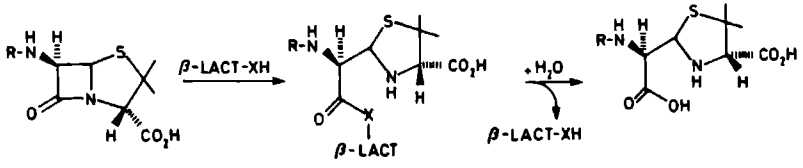
Fig.2 : Stereoprojection of penicillin (upper left) and acyl-D-alanyl-D-alanine (lower right). The arrows indicate the amide bonds cleaved by the transpeptidase.



This must be caused by the ring strain, which hinders an efficient orbital overlap of the β-lactam carbonyl- and amino-function. Figure 2 shows that the highly reactive CO-N bond in the β-lactam ring of penicillin occupies a spatial position analogous to that of the CO-N bond in acyl-D-alanyl-D-alanine.

Despite of these unique properties of the β-lactam antibiotics, several strains of bacteria have, as indicated already before, built up a resistance against some of them. These bacteria are able to synthesize an enzyme, a so-called β-lactamase, which can hydrolyze the CO-N bond in the β-lactam ring (eq.3).[20,24-26]

(eq 3)



9 penicilloic acid

The product of this reaction, penicilloic acid 9, is inactive as an antibiotic. The activity of the enzyme depends markedly on the nature of the substituents attached to the bicyclic nucleus of the β-lactam compounds.[20,26] As a consequence (semi-)synthetic β-lactam antibiotics bearing substituents that render them unsusceptible to β-lactamase, are clinically very important.

4.3 *BIOSYNTHESIS OF THE PENICILLINS AND CEPHALOSPORINS*

4.3.1 *Introduction*

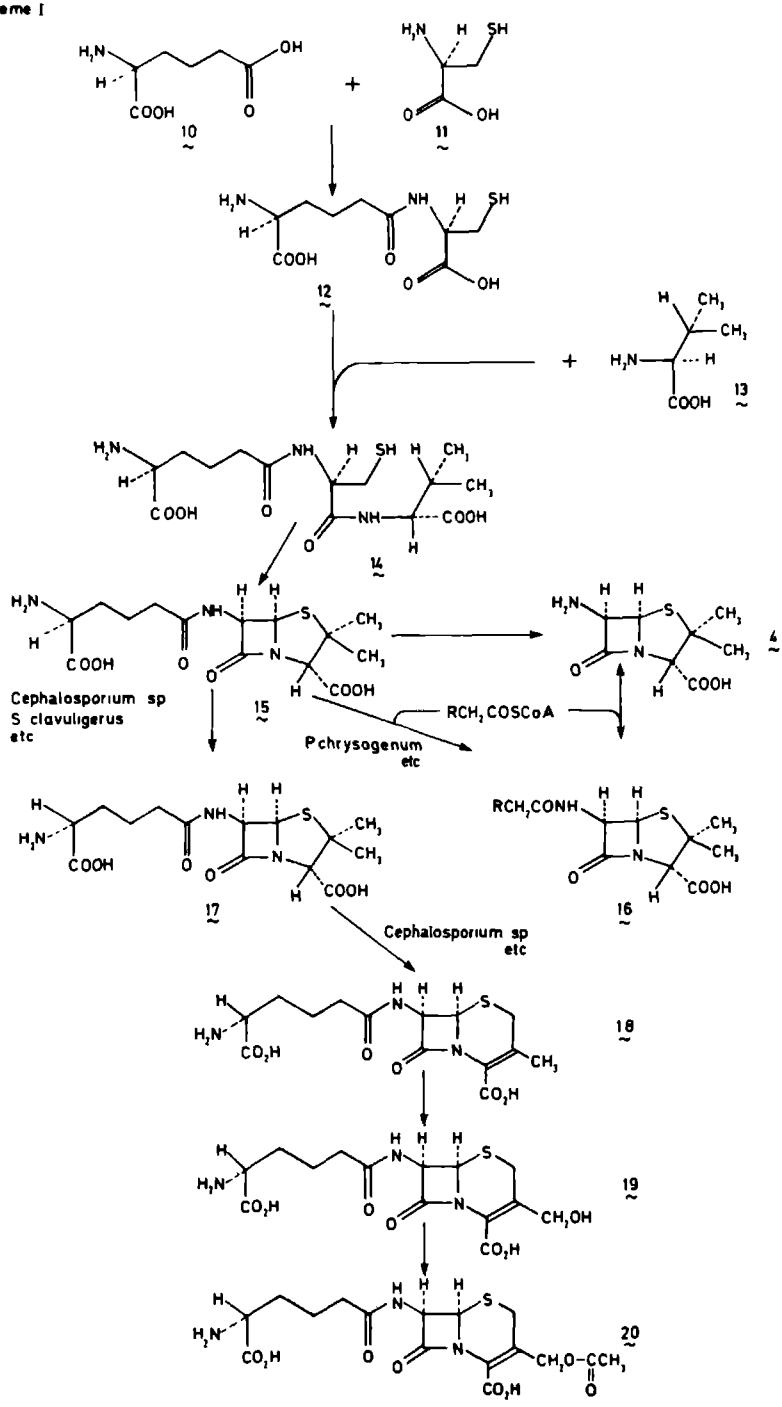
Several excellent reviews on penicillin biosynthesis have appeared in the last decade.[13-15,27-29] This section reflects the current state of affairs. It must be emphasized that many of the facts mentioned in this section were not known when we started our research program (1981; see section 4.4).

4.3.2 *Formation of the penicillins and cephalosporins from the constituent amino acids*

The penicillins and cephalosporins are synthesized from the three amino acids L-α-aminoadipic acid 10, L-cysteine 11 and L-valine 13. The consecutive reactions leading to the antibiotics are depicted in scheme I.

First the dipeptide δ-(L-α-aminoadipyl)-L-cysteine 12 is formed, which then combines with L-valine 13 to form δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine 14. It should be noted that the incorporation of L-valine 13 in the tripeptide 14 is attended with inversion of its chiral centre. The tripeptide 14 is named after its discoverer, Arnstein.[30] The next step is the cyclization of the Arnstein tripeptide 14 to isopenicillin N 15. Subsequently the metabolic fate of isopenicillin N differs depending on the organism.

Scheme I


10
11
12
13
14
15
4
16
17
18
19
20
Cephalosporium sp
S clavuligerus
etc
RCH2COSCoA
P chrysogenum
etc
Cephalosporium sp
etc

In Penicillium chrysogenum, and other fungi exhibiting the Penicillium type of fermentation, isopenicillin N 15 can be converted into one or more penicillins with a monosubstituted acetyl side chain derived from an appropriate monosubstituted acetyl-CoA precursor. Whether this happens by transacylation of isopenicillin N (15 → 16), or deacylation to 6-APA 4 and subsequent reacylation, or via both processes, is not clear yet.

In species showing the Cephalosporium type of fermentation, *e.g.* Cephalosporium acremonium and Streptomyces clavuligerus, the δ-(L-α-aminoadipyl) side-chain of 15 undergoes inversion and the resulting penicillin N 17 is excreted. Part of the penicillin N 17 is converted intracellularly into deacetoxycephalosporin C 18. This ring expansion reaction can be followed by an oxidation of the exocyclic methyl group, affording deacetylcephalosporin C 19. Subsequent acetylation yields cephalosporin C 20.

4.3.3 *Conversion of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine into isopenicillin N*

After the isolation[30] of the Arnstein tripeptide 14 its biochemical conversion into isopenicillin N 15 has been introduced as a presumption,[31] known as the Tripeptide Theory. It was fortified, more than 10 years later, when it was noted that the configurations of the constituent amino acids of 14 appear to match those of the corresponding units in 15.[32,33] A significant breakthrough was achieved in this field of research by the development of reliable cell-free systems. This enabled Abraham's[17,34] and Baldwin's[35] group to deliver further support for the Tripeptide Theory.

Some years later the enzyme isopenicillin N synthetase (IPNS) that catalyzes the conversion of 14 into 15 was purified.[36,37] Subsequently, the gene encoding IPNS in Cephalosporium acremonium was isolated and, after cloning it into an E. coli expression vector, inserted into E. coli.[38] The recombinant E. coli thus obtained, produced IPNS as the major protein of the cell (~ 20% of the cell protein). This may be considered as a highlight in β-lactam research.

Before this major achievement was accomplished, several problems concerning the conversion of 14 into 15 were studied by means of cell-free systems, prepared by osmotic lysis of protoplasts of Cephalosporium acremonium,[17] or purified IPNS. This approach afforded a number of new insights.

So it was established that the enzyme uses dioxygen as a cosubstrate for the oxidative cyclization of 14 to 15 : one mol of dioxygen is used for the synthesis of one mol of 15, which is the stoichiometry expected for the loss of four hydrogen atoms from 14 (eq.4).[39]

$$\text{(eq 4)}\quad \text{(LLD)-ACV}(\underset{\sim}{14}) + O_2 \xrightarrow[\text{L-ascorbate, catalase}]{Fe^{2+},\ \text{DTT}} \text{Isopenicillin N}(\underset{\sim}{15}) + 2H_2O$$

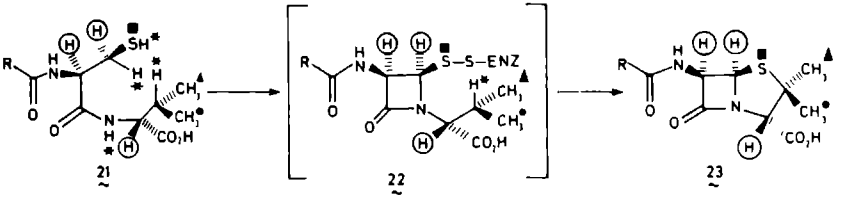
To stimulate activity four cofactors are used in the incubation of the (LLD)-ACV 14 with a cell-free system or IPNS. It seems that of these cofactors only ferrous ion has a primary function, whereas the other three, L-ascorbate, DTT and catalase, play a role in maintaining both the enzyme and its substrate in their active (=thiol) form.[28]

To a certain extent the enzyme tolerates some structural

variations in the substrate (see also section 4.3.4). As far as the α-aminoadipyl side-chain is concerned, the minimal structural requirement for N-acyl-L-cysteinyl-D-valine peptides to be converted into penicillin products by IPNS is that the N-acyl group has a six carbon or equivalent chain, terminating in a carboxyl group.[40,41] This points to a distance between the binding site and the active site (which is probably bound to the cysteinyl thiol group of the substrate) which corresponds with the distance between the carboxyl group in the side-chain and the thiol group in a substrate.[28,40] If the substrate does not fulfil the before-mentioned criteria it is not at all or only slowly converted into a penicillin, as was demonstrated by the slow formation of penicillin V (eq. 5; 23, R=$PhOCH_2$) and penicillin G (eq. 5; 23, R=$PhCH_2$) from the corresponding dipeptide derivatives (21, R=$PhOCH_2$ or $PhCH_2$, respectively).[42]

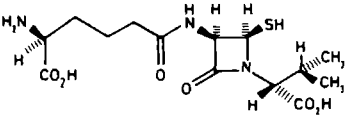
Various features with respect to the stereochemical course of the oxidative condensation have been determined and are summarized in eq.5.

(eq 5)



Although a free or enzyme-bound intermediate has not yet been detected,[43] Baldwin succeeded in finding direct evidence for a stepwise ring closure involving initial β-lactam formation, *i.e.* 21→22.[44] This finding and the presence of at least one cysteinyl thiol in IPNS, whose blockade inhibits the enzyme's activity,[45] point to the formation of an enzyme-bound monocyclic

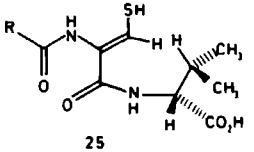
β-lactam intermediate like 22.[27,28] Attempts to use β-lactam 24 as a substrate of isopenicillin N synthetase were unsuccessful as the pH-stability range of 24 and that of the enzyme do not overlap sufficiently.[28,46]



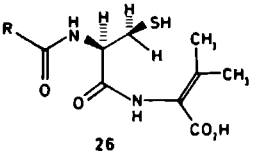
24

It has been shown[45] that transfer of sulphur between the Arnstein tripeptide molecules (21, R=δ-(L-α-aminoadipyl)) does not occur during their conversion into isopenicillin N (23, R=δ-(L-α-aminoadipyl)), which indicates that the integrity of the cysteinyl C(3)-S bond is maintained.

The β-lactam formation occurs with complete retention of the cysteinyl 3-pro-R-hydrogen and with complete loss of the cysteinyl 3-pro-S-hydrogen (H*).[47] Similarly, the closure of the thiazolidine ring by C-S bond formation (22→23) proceeds with complete retention of the valyl C-3 stereochemistry.[48] It has been demonstrated clearly[13-15,27-29,45,47-53] that all of the atoms of 21 except those which have been asterisked, are incorporated into 23. The observation that the encircled protons are retained, rules out mechanisms involving the formation of free intermediates containing a dehydrocysteine (25) or a dehydrovaline (26) residue during β-lactam and thiazolidine ring formation, respectively.



25



26

It remains to be explained by which mechanism 14 is converted into 15. The next section will be concerned with this question.

4.3.4 *Mechanism for the oxidation of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine*

4.3.4.1 *Introduction*

Although the biogenetic precursors of penicillins and cephalosporins were established with relative ease, attempts to elucidate by which mechanism the fused ring systems are formed have met with considerable difficulties. A number of plausible intermediates have been proposed on the basis of chemically feasible mechanisms for the formation of the penam nucleus.[13-15] However, over the past few years most of these hypothetical intermediates have been precluded on the basis of isotopic labelling studies (see 4.3.3)[15] or, alternatively, by direct assessment of the synthesized "intermediates" as substrates for cell-free isopenicillin N synthesizing systems.[15,28,54]

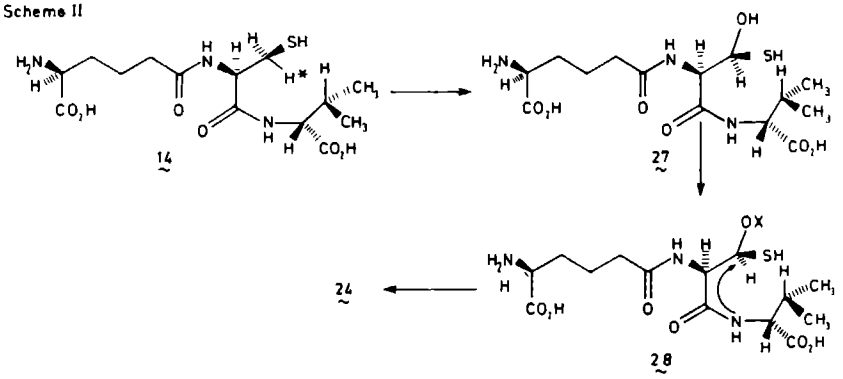
The intention of this section is to summarize the prevailing ideas with regard to the mechanistic course of the cyclization of the Arnstein tripeptide 14, affording isopenicillin N 15.

4.3.4.2 *β-Lactam ring formation*

Despite intensive investigations the intimate details of the formation of the β-lactam ring have not yet been elucidated completely. Since a large number of labelling experiments have set fairly rigid requirements for any proposed biosynthetic mechanism, only four general mechanisms, which are in accord with present experimental data, have remained.

In one of them, the intermediacy of an activated alcohol is proposed (scheme II).[15]

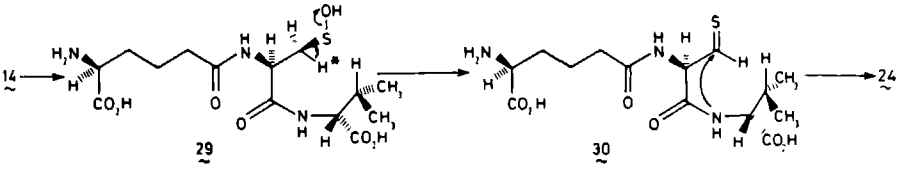
Scheme II



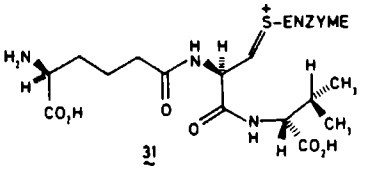
Stereospecific hydroxylation at C-3 in the cysteinyl residue of 14 followed by activation of the hydroxyl group, 27 → 28, and intramolecular displacement of the activated hydroxyl group, 28 → 24, may yield the β-lactam ring. As C-N bond formation proceeds with retention of configuration,[47] both hydroxylation and intramolecular displacement would have to occur either with inversion or with retention of configuration. However, while most hydroxylations at aliphatic carbon occur with retention, the intramolecular displacement is expected to occur with inversion. Consequently, this mechanism is rather improbable as it should involve unusual stereochemistry. Besides, it must be noticed that at least 24 must be bound to the enzyme by its thiol functionality, since such a compound is not stable as a free intermediate[28,46] under the incubation conditions.

A second proposal involves oxidation at sulfur, resulting in the formation of the thioaldehyde intermediate 30 (scheme III).[15] This mechanism is contradicted by Baldwin's observation[46a,c] that under the incubation conditions 24 is not stable and is converted

Scheme III



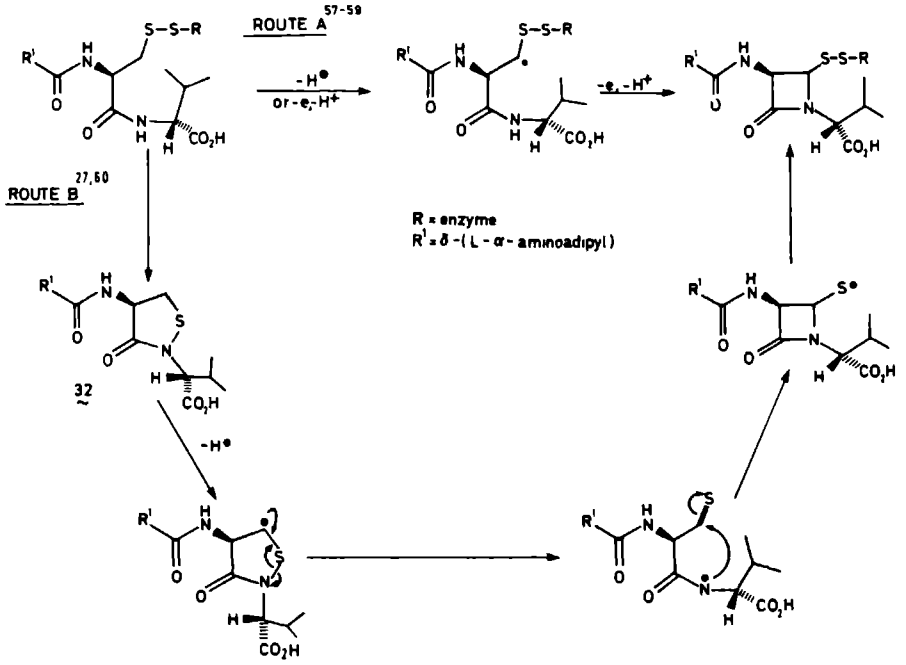
into 30, which subsequently reacts very fast intermolecularly with a nucleophile. A slight modification of the mechanism depicted in scheme III, which meets this difficulty, involves the intermediacy of 31, an enzyme-bound thioaldehyde[55] which is formed by a Pummerer reaction[56] of an appropriate sulfoxide and subsequently converted into an enzyme-bound β-lactam like 22. This fits in with all available experimental data.



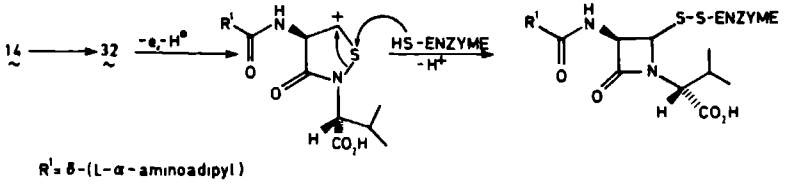
The feasibility of such a mechanism has been demonstrated by some model reactions.[55]

As a third possibility, a free radical intermediate has been considered (scheme IV).[27,57-60] Even though proceeding in low yields, reactions in model systems proved the feasibility of route A.[57,59] Although the chemical validity of the other route (B) could not be established,[27,60] a modified version of this mechanism (scheme V), also involving the intermediacy of isothiazolidinone 32, was supported by experiments in model systems.[61]

Scheme IV



Scheme V



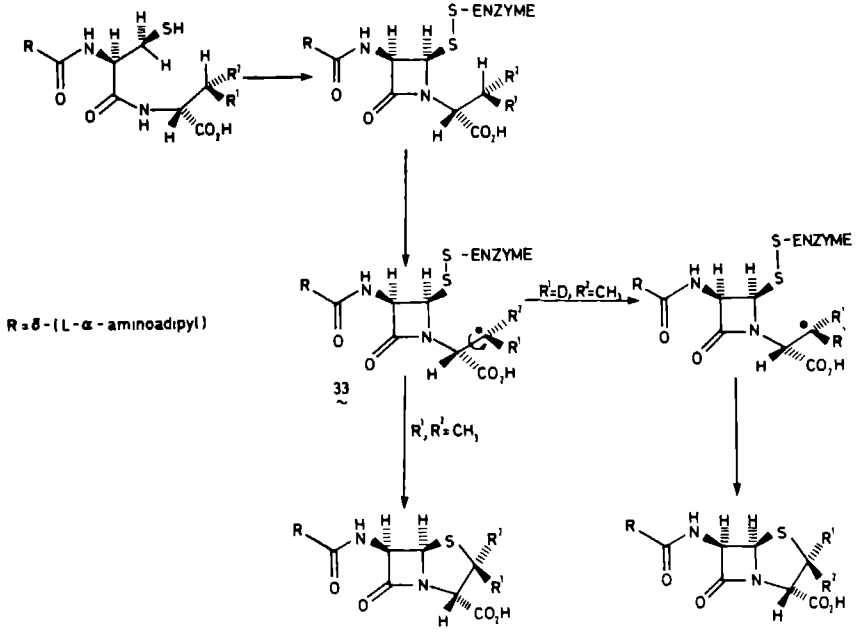
Finally, the fourth alternative, *i.e.* a route via an N-hydroxy tripeptide, will be discussed in section 4.4.

4.3.4.3 *Thiazolidine ring formation*

In contrast with the uncertainty about the mechanistic pathway of β-lactam ring formation, all information concerning thiazolidine ring formation points to a mechanism involving a free radical intermediate (scheme VI; R^1=R^2=Me). Attempts to model this ring closure by using artificially reactive systems were not always successful.[62-65]

On the other hand, convincing data supporting the intermediacy of a free radical intermediate were acquired, especially during the last few years, from experiments with structural analogues of the Arnstein tripeptide 14, characterized by substantial variations of the valyl moiety.[28,66-74] It was shown that isopenicillin N synthetase is able to accept such modified tripeptides and transform them into substituted derivatives of isopenicillin N. Besides,

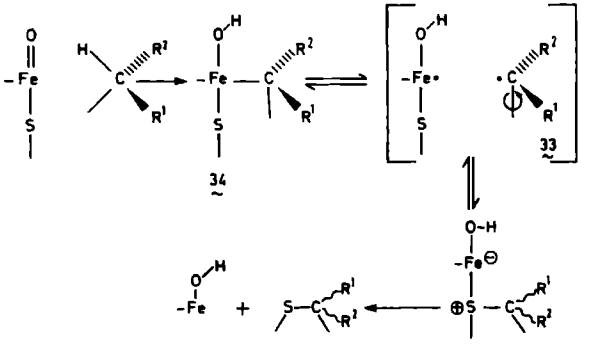
Scheme VI



dependent on the nature of the substituents R^1 and R^2 (scheme VI), the formation of new bicyclic β-lactams, containing a six- or even seven-membered heterocycle, was observed in some cases. Both the regio- and stereochemical course of the reactions were in accord with the formation of the free radical intermediate 33. From the results it is evident that the active site topology of the enzyme puts strong restraints on the rotational motion in 33 (see arrow) : only when R^1 or R^2 is deuterium, rotation is possible.[28,48,69]

Furthermore, as a working hypothesis an intermediate organoiron species 34 (scheme VII) has been suggested.[28,74,75] Such a species might undergo homolysis and subsequent recombination, affording the bicyclic product (see also section 4.8.8).

Scheme VII

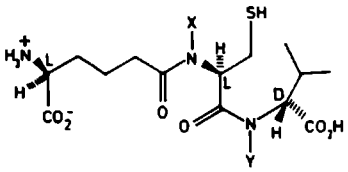


The attractiveness of this rationale is that it explains the function of the ferrous ion, one of the cofactors used in incubations.[28]

4.4 *N-HYDROXY ARNSTEIN TRIPEPTIDES AS INTERMEDIATES IN PENICILLIN BIOSYNTHESIS : A POSTULATE*

Since the formation of the β-lactam ring as well as the thiazolidine ring is an oxidative process, it has been proposed

that the N-hydroxy peptides 35-37 might be involved as intermediates in penicillin biosynthesis.



35 X=H, Y=OH

36 X=Y=OH

37 X=OH, Y=H

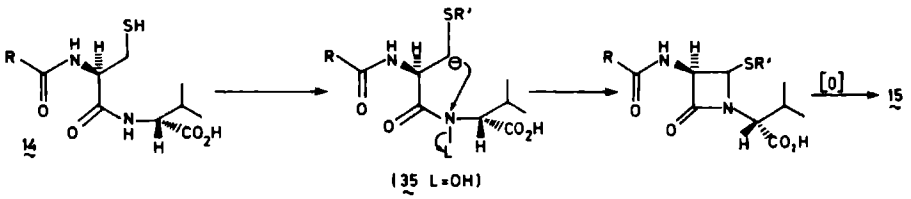
It has already been mentioned that oxidation of nitrogen is an activating step in the metabolism of many nitrogen containing compounds (see section 1.1). Besides, it has been demonstrated that many natural hydroxamic acids arise from corresponding amides by direct oxidation (see section 2.4). A substantial number of natural products containing one or more oxidized peptide bonds, -C(O)-N(OH)-, have been found in nature, some of them being produced by species belonging to the genus Penicillium.[76-78] These compounds are of continuing interest owing to their biological activity[76,77,79] and their proposed intermediacy in the biosynthesis of microbial metabolites.[80,81]

As was discussed in section 4.3, chiral labelling studies have placed severe restrictions on any mechanism proposed for the biochemical oxidation of the Arnstein tripeptide to the penam nucleus. Mechanisms, which satisfy the requirements for retention of the α-S and β-pro-R protons of the cysteine residue and the α-hydrogen of the (D)-valine residue, can however be formulated for the conversion of each of the N-hydroxy Arnstein tripeptides 35-37 into isopenicillin N. These mechanisms will now pass in review.

Oxidation at the nitrogen of the valine residue in the tripeptide 14, followed by nucleophilic displacement by an anion

generated at the β-carbon atom of the cysteine fragment has been proposed by Birch and Smith[82] (scheme VIII).

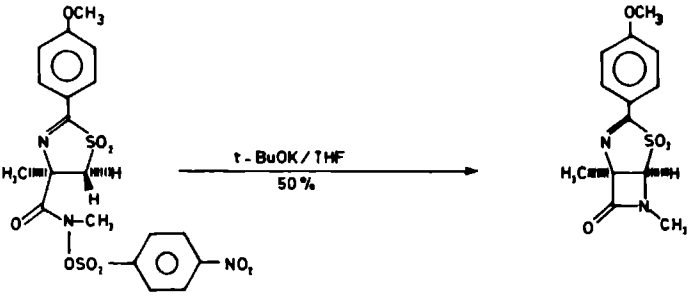
Scheme VIII



(35 L=OH)

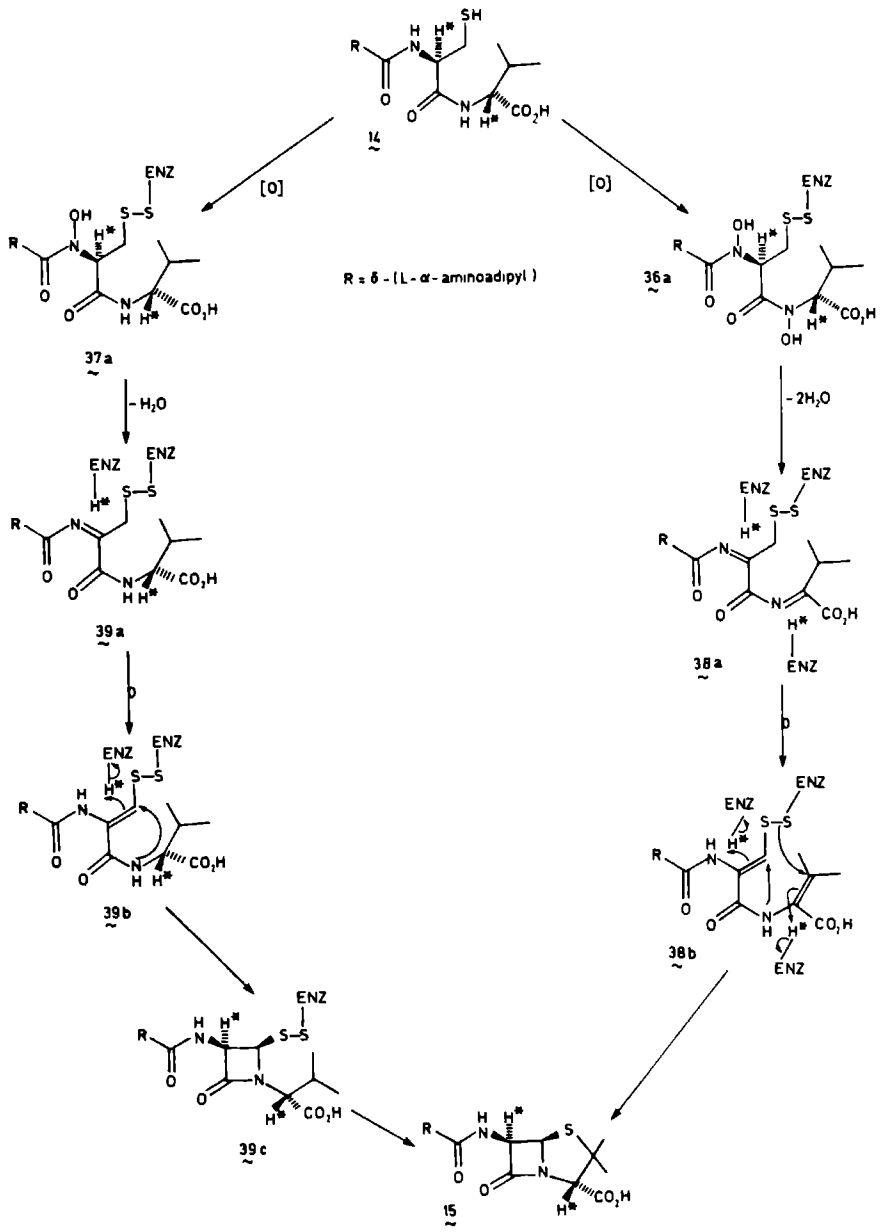
Scott[81] worked out this postulate by proposing the formation of a hydroxamic acid intermediate 35 (scheme VIII; L=OH). This hypothesis was supported by the model chemical reaction which is depicted in eq.6.[81]

(eq.6)



The two other N-hydroxy peptides 36 and 37 were proposed as intermediates by our group (see e.g. ref. 83). The mechanisms depicted in scheme IX may account for the eventual conversion of 36 and 37 into isopenicillin N. In the first step water is eliminated to give 38a resp. 39a. Then in both compounds the double bonds rearrange to give the dehydro-intermediates 38b resp. 39b. Intramolecular attack on the double bonds in 38b by the valine nitrogen atom and the sulfur atom yields isopenicillin N. In 39b only one double bond is present. Nucleophilic attack of the valine

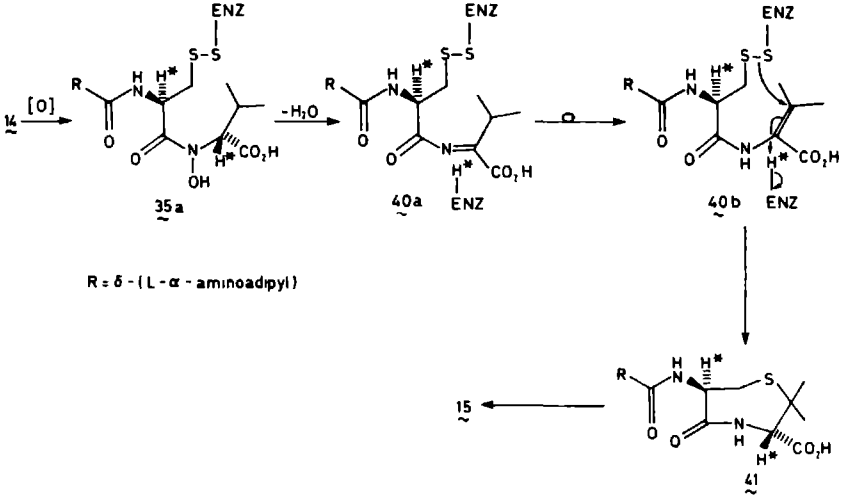
Scheme IX



nitrogen atom on this double bond gives the monocyclic intermediate 39c, which has to be converted subsequently into isopenicillin N by thiazolidine ring formation via another mechanism. The mechanisms will only be compatible with the results of chiral labelling studies when the α-hydrogens, which are abstracted during the first step, are replaced in the original positions by the enzyme. This condition may be fulfilled if during the whole process the dehydro-intermediates 38 and 39 remain enzyme-bound like depicted in scheme IX. It is worth noting that this assumption is supported in that there is indeed no experimental evidence for the accumulation of any free intermediate during the in vitro enzymatic transformation of the Arnstein tripeptide to isopenicillin N.

It has also been suggested that for the potential conversion of 35 into isopenicillin N an alternative mechanism, analogous to those pictured in scheme IX, can be formulated (scheme X).

Scheme X



However, since the intermediacy of 41 appeared to be in conflict with Baldwin's observation[44c,54] that 41 does not behave as a substrate of the IPNS, this idea was abandoned.

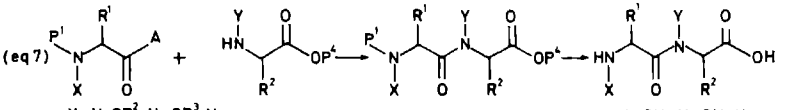
To test the validity of the postulate the N-hydroxy peptides 35-37 had to be synthesized. Subsequently direct assessment of the synthesized 'intermediates' as substrates for a cell-free isopenicillin N synthesizing system should prove whether they are converted into isopenicillin N or not. So, we aimed at the total synthesis of the three N-hydroxy peptides 35-37. The results of this investigation are presented in the next part of this chapter.

4.5 *GENERAL REMARKS WITH RESPECT TO THE SYNTHESIS OF N-HYDROXY PEPTIDES*

Before describing the results of our attempts to synthesize the N-hydroxy peptides 35-37 some general information concerning the synthesis of N-hydroxy peptides is given.[84-87]

Although N-oxidation plays a role in the in vivo metabolism of amines and amides, N-hydroxy peptides are not yet accessible by in vitro oxidation of the peptide bond.[88] Therefore unambiguous synthesis of N-hydroxy peptides, just as that of 'normal' peptides, must go through three stages : synthesis of adequately protected N-hydroxy amino acids, formation of the N-hydroxy peptide bond, and finally selective removal of one or more of the protecting groups.

(eq 7)



X = H, OP^2, Y = OP^3, H
A = activating group
P = protecting group

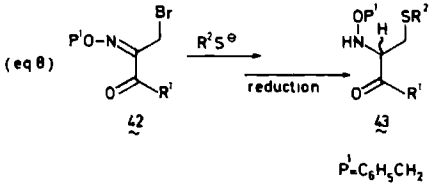
X = H, OH, Y = OH, H

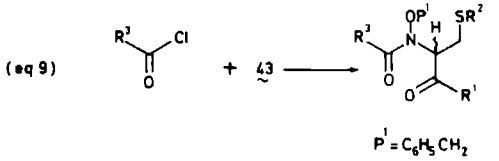
This process is illustrated in eq.7 for the synthesis of a dipeptide having one (X or Y = OH) or two (X = Y = OH) N-hydroxy functions. In contrast to 'normal' peptide synthesis there are mainly two differences.

First, due to the inductive effect of oxygen the nucleophilicity of the hydroxylamine function, and even more so of the O-benzyl derivative (vide infra), is decreased. Consequently coupling requires highly activated carboxyl groups.[85] Secondly, for unambiguous N-acylation the use of O-protected N-hydroxy amino acid derivatives is often required. For this purpose the benzyl group was found to be the most suitable protecting group,[89] although it causes increased steric hindrance and decreased nucleophilicity compared to the corresponding free hydroxylamine function. It can be removed by hydrogenolysis,[85a,b] by treatment with boron tris-(trifluoroacetate),[85a,b] or, as shown only recently,[90] by treatment with sodium in liquid ammonia.

A special problem anticipated in the synthesis of the N-hydroxy Arnstein tripeptides concerns the synthesis of the N-hydroxy cysteine residue. Due to the presence of the easily oxidizable sulfur atom this residue is not accessible by oxidation of cysteine. Eq.8 and 9 show how we intended to circumvent the anticipated problems.

The strategy employed involved nucleophilic substitution of bromine in 42 by a suitable mercaptide ion. Then, by using a method developed in our laboratory,[91-94] reduction of the oxime double bond should yield racemic 43. In order to achieve selective N-acylation we preferred the use of O-protected N-hydroxy amino acid derivatives. Consequently a highly reactive carboxylic acid





derivative was needed, *i.e.* an acid chloride (eq.9).

4.6 *ATTEMPTED SYNTHESIS OF N-[δ-(L-α-AMINOADIPYL)-L-CYSTEINYL]-N-HYDROXY-D-VALINE* (35)

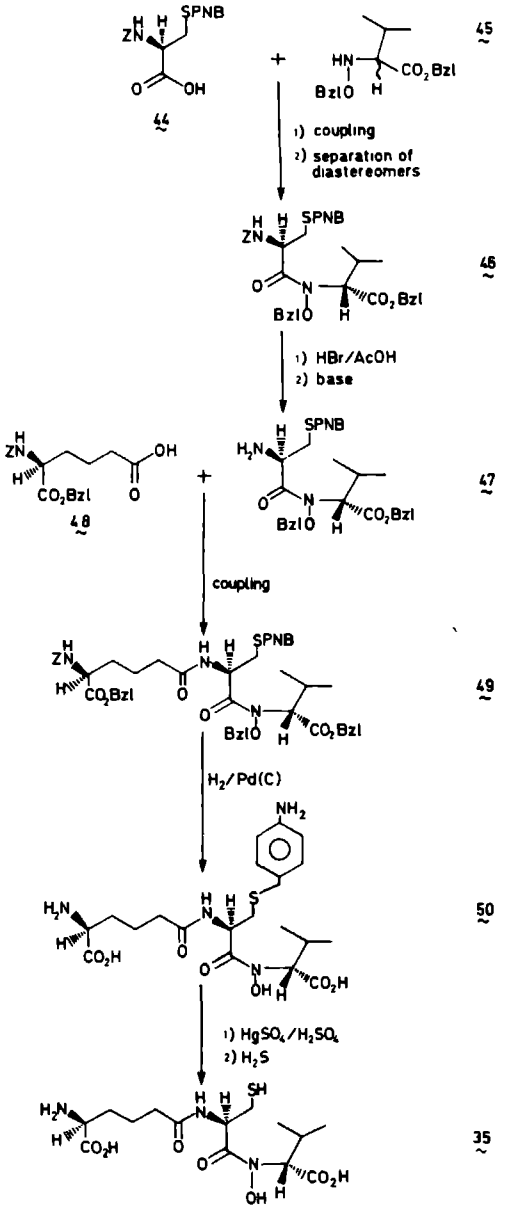
4.6.1 *Introduction*

Our first goal was the synthesis of compound 35. Taking into account the special demands made upon N-hydroxy peptide synthesis we designed the synthesis plan depicted in scheme XI.

It was agreed that Prof. Dr. J.E. Baldwin (Oxford University) would supply us with an amount of compound 48. So, in order to reduce the number of deprotection steps to a minimum, benzyl groups were used for the protection of the functional groups of 47, with the exception of the thiol functionality. The choice of the S-protecting group deserves some comment.

It was recognized that only rather mild conditions could be used for the removal of the protecting groups. Basic conditions would be mischievous because it is well documented that compounds like 49 and 35 undergo readily elimination of benzyl alcohol and H_2O, respectively[84] (see section 2.2.3). An acid-labile S-protecting group would not be compatible with the conditions

Scheme XI


SPNB
45
44
1) coupling
2) separation of diastereomers
46
1) HBr/AcOH
2) base
47
48
coupling
49
$H_2/Pd(C)$
50
1) $HgSO_4/H_2SO_4$
2) H_2S
35

(HBr/AcOH) used for the selective removal of the benzyloxycarbonyl-group, 46 → 47, at an early stage of the synthesis. So, we chose for a two-step deprotection of compound 49.

First, all the benzyl groups would be removed by catalytic hydrogenolysis. It was realized that poisoning of the catalyst might be a problem. However, it is well-known[95] that this can be prevented by modifying the catalyst or adaptation of the reaction conditions. The second step would involve a treatment with Hopkins reagent[96] ($HgSO_4/H_2SO_4$) to remove the remaining p-aminobenzyl group from the sulfur atom.[97] Finally, decomposition[97] of the resulting mercury complex with H_2S should yield the free peptide 35.

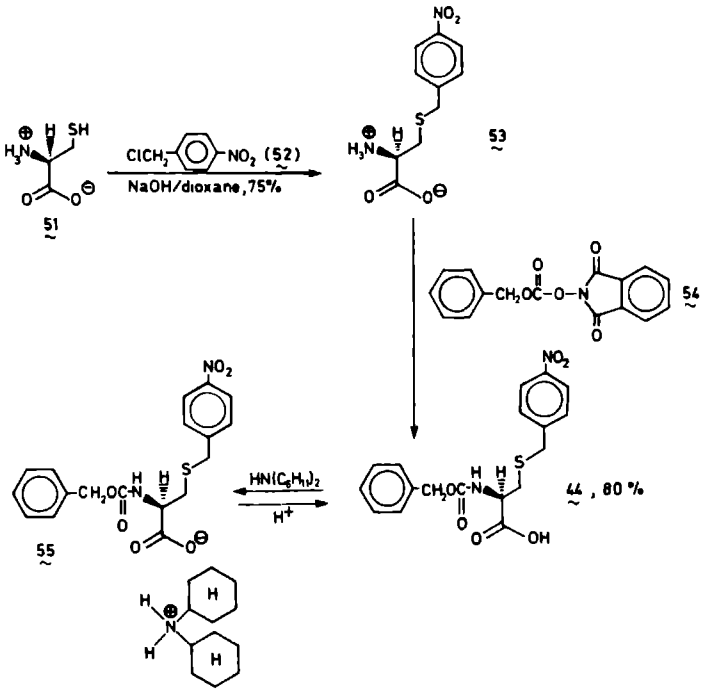
Before reporting the results of our attempts to construct tripeptide 35 according to scheme XI, attention will be payed to the synthesis of the starting materials. Several methods for the synthesis of compound 48 have already been published, see *e.g.* ref. 98. The synthesis of the compounds 44 and 45 will be the subject of the next two sections.

4.6.2 *Synthesis of N-benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteine*

The synthesis of 44 was a two-step procedure (scheme XII). The first step involved protection of the sulfur atom of L-cysteine 51; nucleophilic attack on 4-nitrobenzyl chloride 52 afforded compound 53 in good yield.[99]

Subsequently the benzyloxycarbonyl group was introduced, using the N-hydroxyphtalimide derivative 54,[100] for the protection of the amino group. Purification of the product 44 was achieved by converting it into its dicyclohexylamine salt 55,[101] which could be crystallized from 2-propanol.

Scheme XII



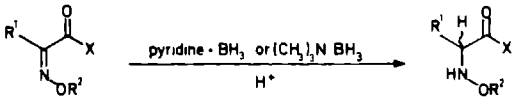
4.6.3 *Synthesis of N-benzyloxy-D,L-valine benzyl ester*

Most syntheses reported so far for N-hydroxy-α-amino acid derivatives are laborious, give poor yields or have limited application.[102,103] The method of choice for the synthesis of N-hydroxy-α-amino acid derivatives is reduction of the corresponding oximino compounds (eq.10). These precursors are readily available by reaction of hydroxylamines with α-keto acid derivatives. For the reduction mild reagents have to be employed to avoid over-reduction to the corresponding α-amino acids. Cyanoborohydrides meet this requirement; they have been used[103-105] for the reduction (50-75% yield) of α-oximino carboxylic acids (eq.10; R^2=H, X=OH). However, the reaction fails when esters or amides (eq.10; X=OR^3 or NHR^3)

are used because they compete with the oxime for protonation.[83]

The use of a borane-pyridine complex as the reducing agent solves this problem (eq.10).[91]

(eq 10)[91,94]

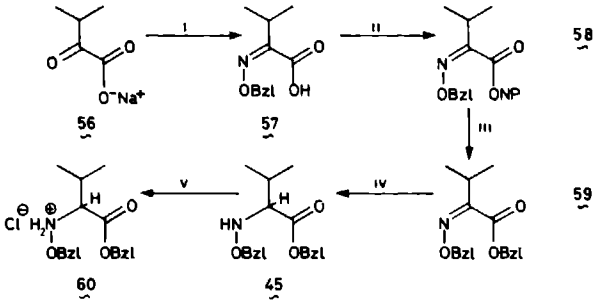


X = OR³, NHR³ , R² = H, C₆H₅ CH₂

When even stronger acidic reaction conditions are needed to protonate the oxime function, an amine-borane complex (*e.g.* $(CH_3)_3N.BH_3$) can be used, because it is more acid-stable than the borane-pyridine complex. It has been employed with esters, amides,[92] p-nitrophenyl esters,[93] and for the synthesis of N-hydroxy tryptophane.[94]

Whereas this method seems to be of general applicability, its drawback is the formation of racemic mixtures. This didn't keep us from using this method for the synthesis of 45 (scheme XIII).

Scheme XIII



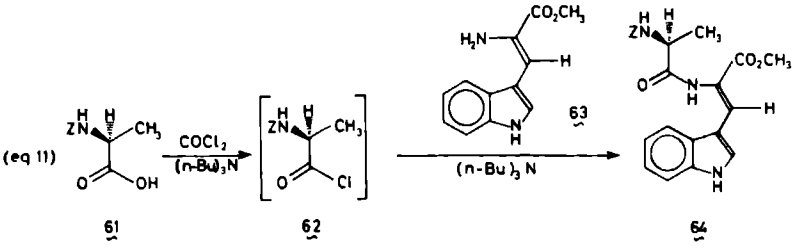
i $C_6H_5CH_2ONH_2$·HCl H_2O 100%
ii 4-O_2N-C_6H_4OH DCC EtOAc 54%
iii $C_6H_5CH_2OH$ DBU CH_2Cl_2 86%
iv $(CH_3)_3N$·BH_3HCl Et_2O 65%
v HCl Et_2O 91%

The preparation of 58 from 56 has been described before.[93]

Transesterification of 58 to 59 proceeded smoothly, affording crude 59 in quantitative yield. Crude 59 could be used in the next step, involving reduction of the double bond 59 → 45, without affecting the yield. Thus, on reduction of 59 with $(CH_3)_3N.BH_3$ in anhydrous ether, saturated with HCl, compound 45 was obtained in 65% yield after column chromatography. Ether instead of an alcohol was used as solvent in this reaction to avoid possible transesterification. A slight impurity still present after the procedure could be removed by converting the desired compound 45 into its hydrochloride salt 60. In contrast with the free base 45, the salt 60 offers the advantage that it can be stored for some time without significant decomposition.

4.6.4 *Attempted synthesis of N-[S-(4-nitrobenzyl)-L-cysteinyl]-N-benzyloxy-D-valine benzyl ester*

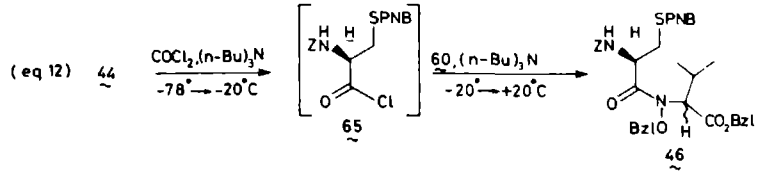
After completion of the synthesis of 44 and 45, we had to consider means of achieving the coupling of these two fragments. From previous literature reports[85a,b] it was clear that a highly activated carboxylic acid derivative was required because of the low nucleophilicity of the N-benzyloxy amino acid derivative 45. The acid chloride or mixed anhydride method seemed to be most suited for this purpose. Since the acid chlorides of amino acids



61 62 63 64

are usually difficult to prepare, we became intrigued by a report[106] mentioning the use of phosgene for the activation of an amino acid (eq.11). It must be emphasized that the authors[106] did not isolate and characterize the acid chloride 62: the activated compound, which was used in a two-fold excess, was in situ coupled with 63 to yield 64 (58%, based on 63).

We employed this method in the coupling of 44 and 45 (eq.12), using only a slight excess of 44.

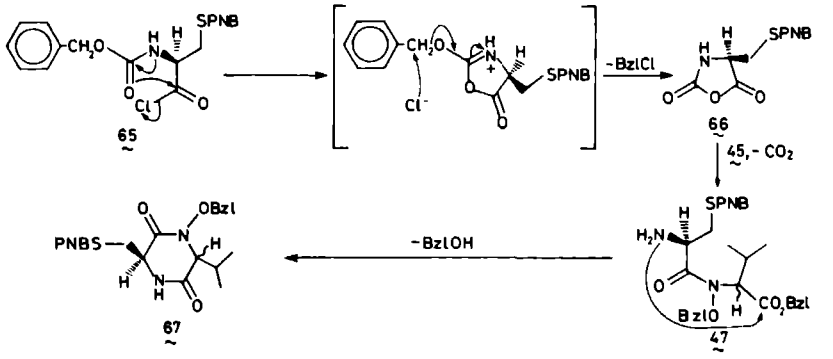


After column chromatography 46 was isolated as a mixture of diastereomers, but the yield was disappointingly low (25% based on 60). Probably the low yield is due to decomposition of the intermediate acid chloride 65, a supposition which was supported by the presence of starting material 45 as well as many impurities in the reaction mixture as monitored by TLC.

A possible side reaction is depicted in scheme XIV; an intramolecular cyclization may lead to the formation of an N-carboxy-anhydride 66 (a so-called Leuchs anhydride).[107,108] Nucleophilic attack of 45 on compound 66 will yield directly compound 47.

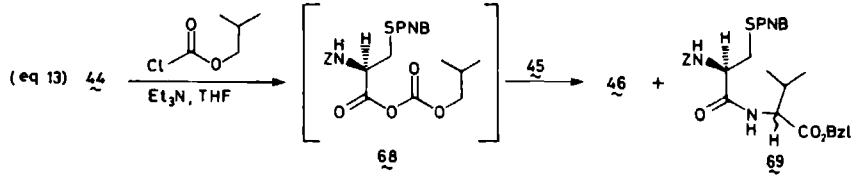
Under the reaction conditions used compound 47 can cyclize to give 67, or, what is even more important, it can compete successfully with 45 for the available acid chloride 65 or anhydride 66, because it is a much better nucleophile.

Scheme XIV

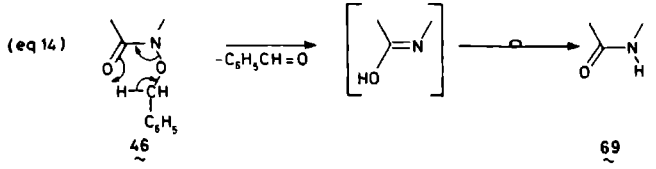


Because of the low yield and the fact that 65 may be prone to racemization, we gave up this method and turned to the mixed anhydride method.

Treatment of 45 with the isobutoxycarbonyl mixed anhydride of 44 (eq.13) resulted in the formation of two products, viz. 46 (1%) and 69 (15%).

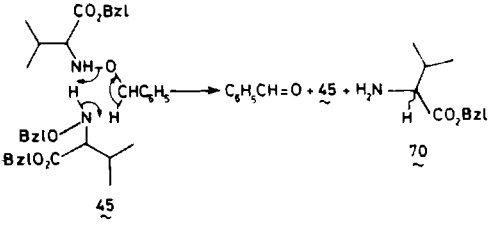


The structure assignment of 69 is based on the ^{1}H-NMR spectrum as well as an independent synthesis. The formation of compound 69 (eq. 13) is difficult to rationalize.



Under the reaction conditions a rearrangement, comparable with the thermal reduction of cyclic hydroxamic acids,[84,109] (46 → 69, eq.14) is unlikely. So, 69 has to result from attack of valine benzyl ester 70 on the activated carboxylic acid 68. Since there are no indications that the starting material 45 was contaminated with valine benzyl ester 70, it must be assumed that 70 arises from disproportionation of 45 in the reaction mixture (eq.15).

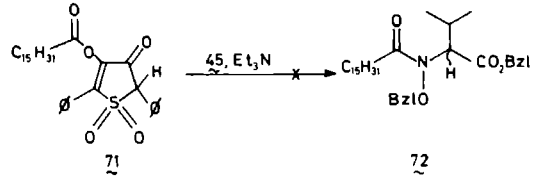
(eq 15)



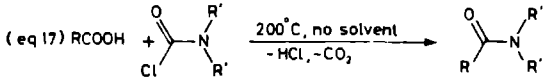
This is a well-known reaction[84,103b,110] of free N-hydroxy-α-amino acids.

In view of this negative result we investigated several other possibilities to accomplish a good coupling reaction between 44 and 45. Activation of carboxylic acid 44 with $POCl_3$,[111] a method which has proven to be of great value in coupling reactions with very poor nucleophiles,[112] gave hardly any product. No reaction at all was observed in a trial experiment with a coupling reagent developed by Steglich[113] (eq.16).

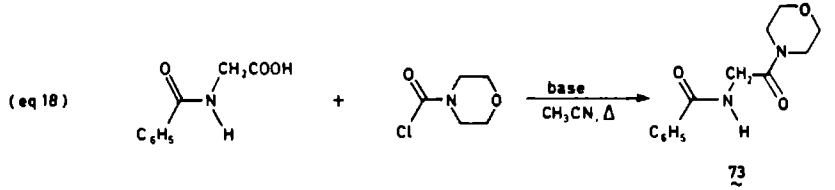
(eq 16)



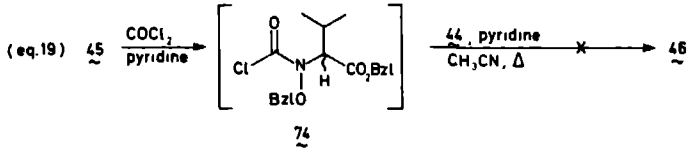
Our next attempt was based on a method (eq.17) described in a Hungarian patent.[114]



The method differs from the others in that the roles of the reactants are reversed; the amine becomes 'activated' and the carboxylic acid function is the nucleophile. Spande[114] applied this method successfully in the synthesis of 73 (eq.18).



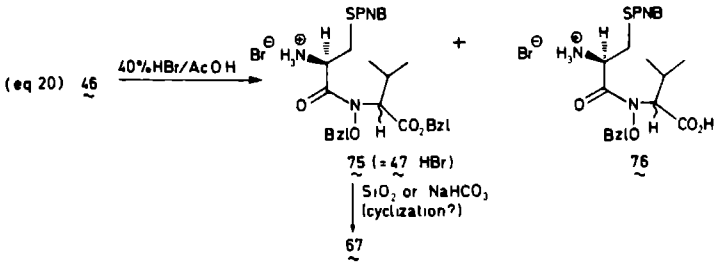
He could avoid excessive heating by using a catalytic amount of a base. However, the procedure failed when applied to 45 (eq.19; the intermediate 74 was not isolated).



Considering all attempts to couple the compounds 44 and 45 (or 60), it seemed that we had to be content with the results of the acid chloride method (eq.12).

The next step in the reaction sequence was the selective removal of the benzyloxycarbonyl group from 46 (46 → 47, scheme XI). It has been shown that the benzyloxycarbonyl group can be removed selectively in the presence of a benzyl ester.[98a,115,116] However, treatment of the dipeptide derivative 46 with HBr in glacial acetic

acid gave a mixture of at least four products (eq.20).



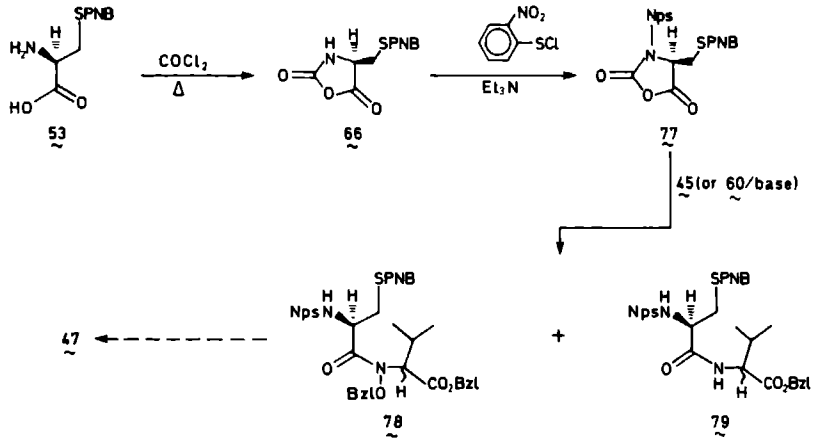
Evidence, though being inconclusive, was obtained that besides 75 also 76 and 67 were formed. The formation of a ninhydrine-negative product, with simultaneous disappearance of a ninhydrine-positive product, was stimulated under the influence of silica gel or sodium hydrogen carbonate (75 → 67?). No conditions could be found which gave only or predominantly 75.

In view of these results another approach for the synthesis of 47 had to be considered. Therefore attention was focused on a coupling reaction involving the use of an N-carboxy anhydride.[107,108,117,118] The principle of this approach is shown in scheme XV. The amino acid 53 is converted into an N-carboxy anhydride (53 → 66), which is provided subsequently with a protecting group on the nitrogen atom (66 → 77). Ring opening by a proper nucleophile (77 → 78) and deprotection (78 → 47) should afford the desired product.

The function of the protecting group (Nps) is to prevent the amine function of 78 from interfering in the contemplated reaction (77 → 78) by making it a poor nucleophile. It has been shown that the o-nitrophenylsulfenyl(Nps)-group is very convenient for this purpose.[119] It can be removed by acidic hydrolysis,[120] by catalytic

desulfurization,[121] or by treatment with nucleophiles.[122] Because of the low nucleophilicity of 45 as compared with a free amine, a crucial role was assigned to this protecting group in our approach (scheme XV).

Scheme XV



Attempts to purify 66 and 77 failed; both compounds decomposed rapidly on trying to recrystallize them. So, crude starting material had to be used in the subsequent steps. The coupling reaction (77 + 45) yielded, besides a lot of starting material 45, a trace of the desired product 78 (<1%) and a small amount of 79. The structure of 79 was confirmed amongst others by an independent synthesis. The formation of 79 can be rationalized by disproportionation of 45 under the reaction conditions used (compare eq.15).

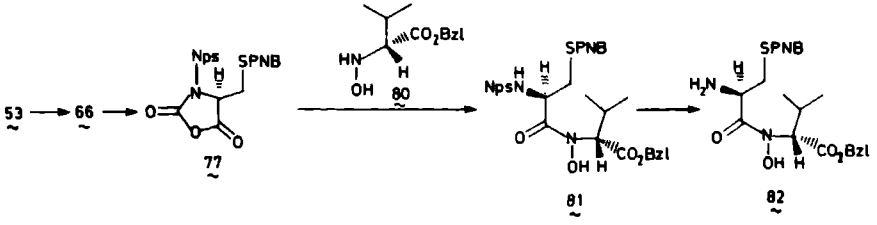
At this point it was realized that the strategy had to be modified. Apparently, the carboxylic acid 44 can not be activated properly for a coupling reaction with N-benzyloxy valine benzyl ester 45. The nucleophilicity of the valine nitrogen is reduced too much by the electron-withdrawing benzyloxy substituent, which may

hamper the reaction also sterically. In our opinion, the failure of the N-carboxy-anhydride approach (scheme XV) is mainly due to steric hindrance.[87b,117a]

4.6.5 *Revised strategy for the synthesis of N-[δ-(L-α-aminoadipyl)-L-cysteinyl]-N-hydroxy-D-valine* (35)

All that remained was using N-hydroxy valine benzyl ester 80 instead of 45 for the coupling with 44, despite of the drawbacks, like rapid disproportionation and O- versus N-acylation, associated with the use of unprotected N-hydroxy amino acids. Encouraged by two reports,[85c,86] describing that selective N-acylation of free N-hydroxy amino acid derivatives can be achieved by employing N-carboxy-anhydrides, we devised the modified synthesis plan given in scheme XVI.

Scheme XVI

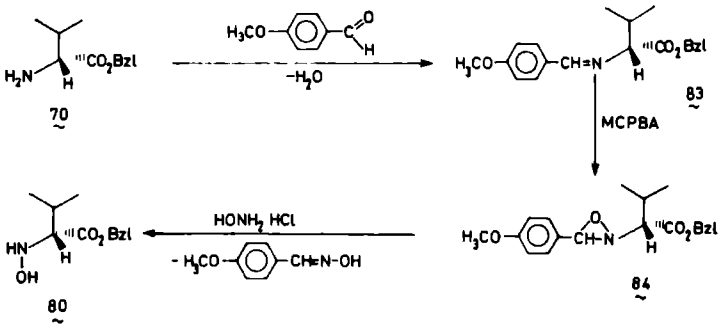


The first purpose was to prepare 80, unreported so far.

Our attention was attracted by a paper[123] that describes the synthesis of optically pure N-hydroxy amino acid esters. This approach features conversion of optically active α-amino acids into the corresponding N-hydroxy-α-amino acids by indirect oxidation without affecting the chiral center. By employing this method it should be possible to prepare 80 according to scheme XVII.

Oxidation of the Schiff base 83 with m-chloroperbenzoic acid or

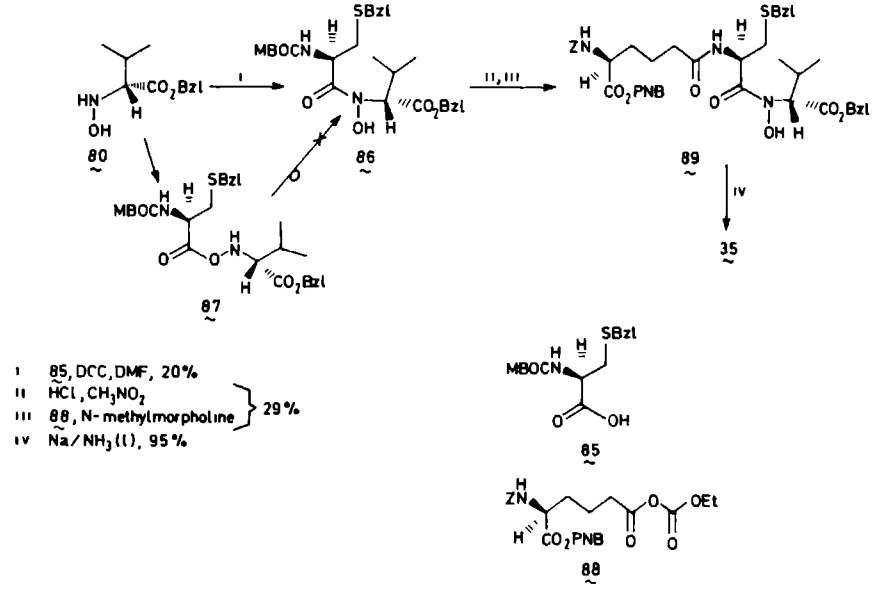
Scheme XVII



monoperphtalic acid, followed by treatment[124] of crude 84 with $HONH_2.HCl$ afforded a mixture of starting material 70 and, probably, 80.

At that time however, a total synthesis of the target compound 35 appeared in the literature.[125] The strategy used shows much resemblance with ours and is summarized in scheme XVIII.

Scheme XVIII

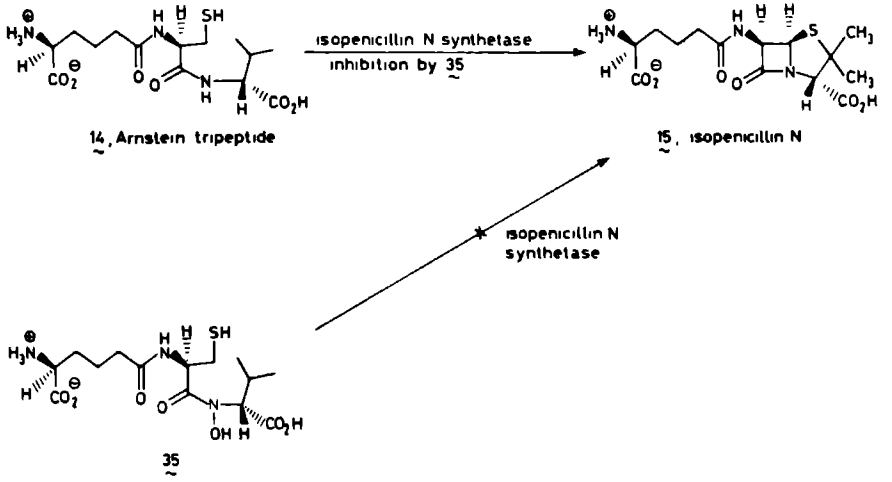


Some notable aspects of this synthesis must be highlighted.

In the first place, the authors had been able, by modifying slightly Chimiak's method,[90,123] to synthesize 80. Then, selective N-acylation of 80 with 85 had been achieved by using DCC as the coupling reagent in DMF. Several other methods gave only O-acylation (80 → 87). In contrast with O-acyl derivatives of simple hydroxylamines,[126] compound 87 could not be rearranged to the N-acylated product 86.[90] It was surprising that the hydroxamic acid function was not affected under the $Na/NH_3(l)$ deprotection conditions (see for comparison eq. 15, section 2.2.3).[127]

Finally, it was shown that 35 was not converted into isopenicillin N by using a partially purified enzyme system derived from homogenized cells of Cephalosporium acremonium (scheme XIX).

Scheme XIX



14, Arnstein tripeptide

15, isopenicillin N

35

These results suggest that the N-hydroxy Arnstein tripeptide 35 is not directly involved as an intermediate in penicillin biosynthesis. On the contrary, the compound was found to inhibit the formation of isopenicillin N from the Arnstein tripeptide completely.

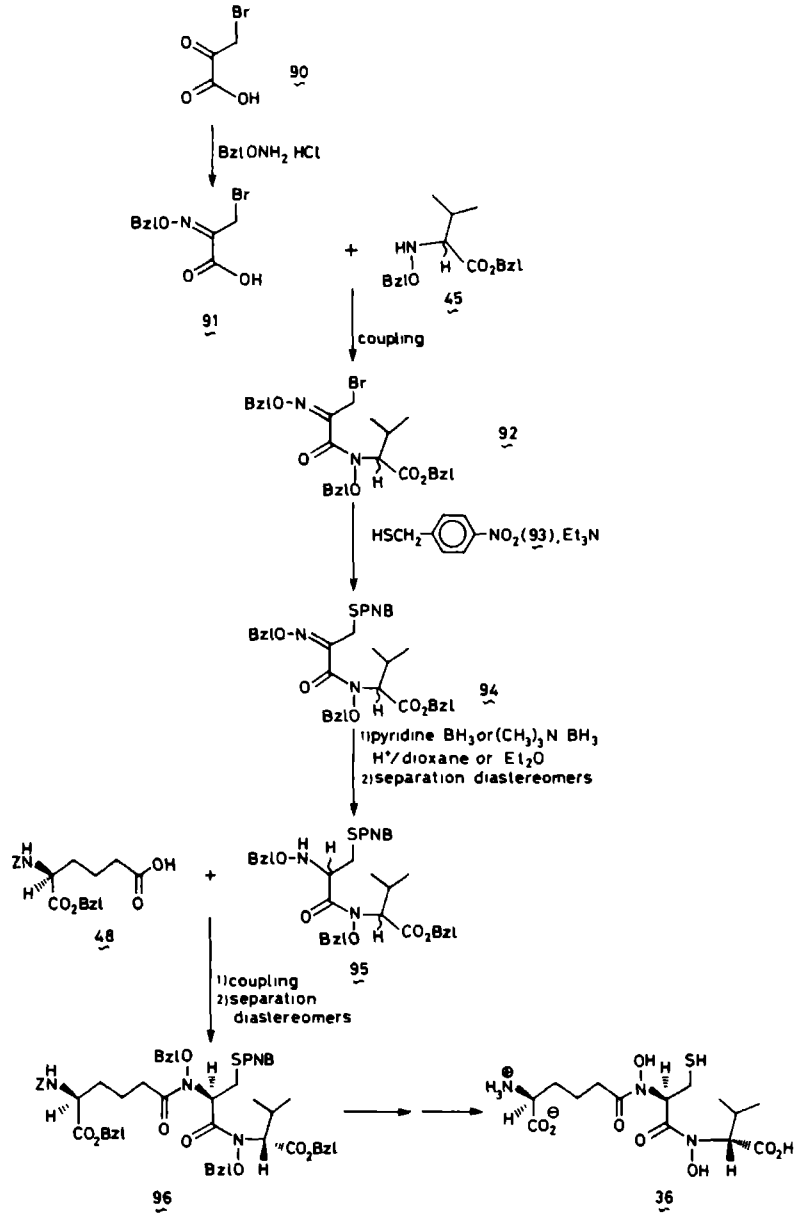
4.7 *ATTEMPTED SYNTHESIS OF N-(N'-[δ-(L-α-AMINOADIPYL)]-N'-HYDROXY-L-CYSTEINYL)-N-HYDROXY-D-VALINE (36)*

4.7.1 Introduction

Prompted by Scott's results (scheme XVIII and XIX)[125] we directed our attention completely to the synthesis of 36 and 37. The most important difference between the synthesis of 35 on the one side and the synthesis of 36 and 37 on the other side arises from the necessity to incorporate N-hydroxy cysteine into the tripeptide. As was explained before (see section 4.5) the only way to achieve this is by an indirect synthesis of the fragment in question, which implicates that the use of a protected cysteine derivative as starting material is avoided. In scheme XX this strategy is worked out for the synthesis of 36.

Coupling of the two fragments 91 and 45 was expected to be straightforward. Nucleophilic displacement of bromine by the 4-nitrobenzyl mercaptide ion (92 → 94), followed by reduction of the double bond should give the protected N,N'-dihydroxy dipeptide 95 as a mixture of four stereoisomers. Separation of diastereomers at this stage would afford two racemic mixtures (RR/SS and RS/SR). After coupling of 95 (RR/SS) with 48, via the acid chloride or mixed anhydride method, again a separation of diastereomers would be necessary. A two-step deprotection of 96 according to the same procedure as described for the synthesis of 35 (scheme XI) should yield 36.

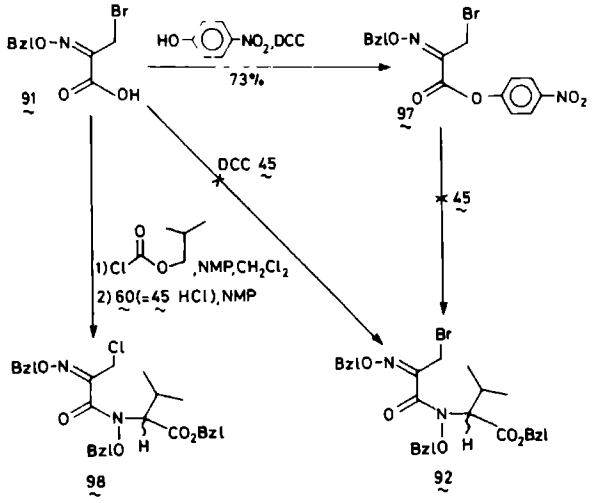
Scheme XX


90
BzlONH2 HCl
91
45
coupling
92
HSCH2 NO2 (93), Et3N
94
1)pyridine BH3 or (CH3)3N BH3
H+/dioxane or Et2O
2)separation diastereomers
48
95
1)coupling
2)separation
diastereomers
96
36

4.7.2 *Synthesis of N-(3-bromo-2-benzyloximino-propanoyl)-N-benzyloxy-D,L-valine benzyl ester*

Condensation of bromopyruvic acid 90 with O-benzylhydroxylamine (scheme XX) afforded the oxime 91 in 88% yield. The synthesis of 92 turned out to be more complicated than was anticipated. Acylation of 45 with the 4-nitrophenyl ester of 91 (*i.e.* 97) failed (scheme XXI).

Scheme XXI



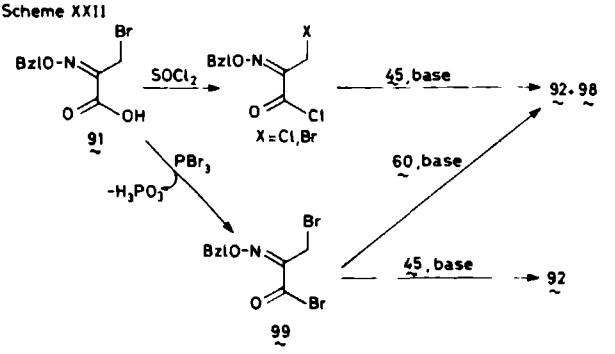
The use of dicyclohexylcarbodiimide as coupling reagent also did not lead to any product formation. Considering Chimiak's experiences with coupling reactions involving mainly rather simple N-benzyloxy amino acid derivatives,[85a,b] our results were actually not surprising. He observed that yields exceeded 30% only when use was made of mixed anhydride or acid chloride activation of the carboxylic acid.

In an orientating experiment conversion of 91 into its isobutoxycarbonyl mixed anhydride, followed by treatment with 60

afforded a coupling product in only 20% yield (scheme XXI). However, the yield could be increased to 45% by applying the reaction conditions recommended by Benoiton (CH_2Cl_2, NMP).[128] From ^{1}H-NMR- and mass spectra it became clear that the product was not the bromine compound 92 but its chlorine analogue 98. Obviously complete displacement of bromine by chloride ions, present in the reaction mixture, had taken place. This side-reaction had to be avoided since it appeared that in the next step the chlorine compound, in contrast with 92, could not be converted smoothly into 94 (see scheme XX).

The same problem, substitution of bromine by chlorine, turned up when the coupling of 91 and 45 was attempted via the acid chloride method (scheme XXII). The mixture of products (92 and 98), consisting mainly of 98, could not be separated by column chromatography. Finally pure 92 could be obtained in 54% yield by using the acid bromide 99 (scheme XXII), and keeping the reaction mixture free from chloride ions. When the hydrochloride salt 60 of N-benzyloxy valine benzyl ester was used in the coupling reaction equal amounts of 92 and 98 were formed. Crude 99 had to be used for the coupling reaction because the compound decomposed rapidly on

Scheme XXII



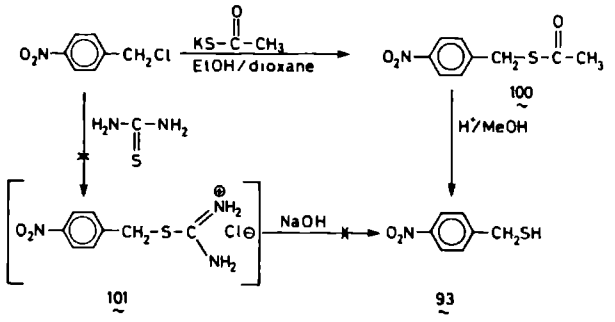
distillation : it was only possible to remove the phosphorous acid and the excess of PBr_3 from the reaction product.

4.7.3 *Attempted synthesis of N-[N'-benzyloxy-S-(4-nitrobenzyl)-D,L-cysteinyl]-N-benzyloxy-D,L-valine benzyl ester*

The synthesis of 94 (scheme XX) proceeded smoothly (55% yield), unless the starting material 92 was contaminated with the corresponding chlorine analogue 98. It is obvious that this has to be attributed to the higher bond strength of the carbon chlorine bond, which makes 98 less susceptible to nucleophilic attack.

The reagent 4-nitrobenzyl mercaptan 93 was readily accessible by treatment of 4-nitrobenzyl chloride with potassium thioacetate and subsequent methanolysis of the resulting thioester 100, the overall yield of 93 being 80% (scheme XXIII).

Scheme XXIII



Application of another method often used for the synthesis of thiols,[129] which should involve the formation of S-(4-nitro)benzyl-isothiouronium chloride 101 in this case, gave no product at all (scheme XXIII).

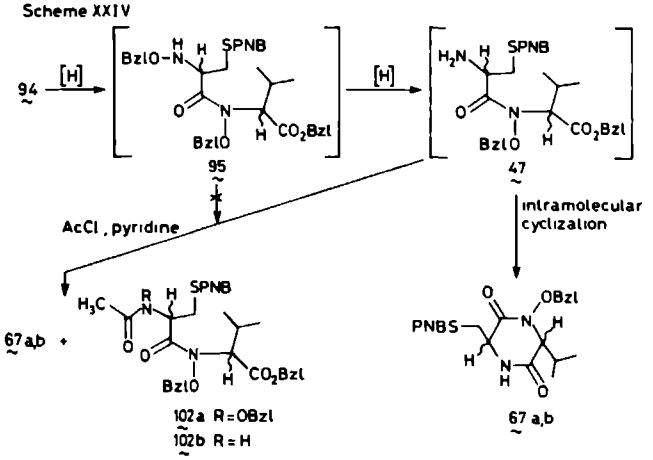
The next step was the reduction of the double bond in 94 in order to obtain the fully protected N,N'-dihydroxy dipeptide 95

(scheme XX). In spite of the rigorous reaction conditions required (the solvent must be saturated with HCl) amine-borane complexes seemed to be most suited for this purpose.[91-94,130] Actually it is the only class of compounds available for the selective reduction of α-oximino esters and amides.[83,84] Most other reducing agents convert oximes and their O-protected derivatives directly into the corresponding primary amines.[131]

Notwithstanding all our efforts we did not succeed in isolating even a minimum amount of the desired product 95. The starting material 94 was consumed only very slowly no matter which reducing agent (pyridine.BH_3 or $(CH_3)_3N.BH_3$) was used. An analytical TLC of the reaction mixture always suggested the presence of at least six, and sometimes even nine, different compounds. After extensive column chromatography minor quantities of two racemic mixtures (a,b : RR/SS and RS/SR) of cyclic compound 67 were obtained (scheme XXIV). Application of the mild reduction method ($NaBH_3CN$/MeOH/pH 3.1) described in chapter V gave no reaction at all.

In one case the excess of reducing agent (pyridine.BH_3) was

Scheme XXIV

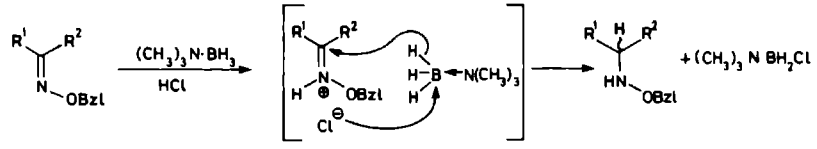


destroyed and removed, after which the crude product was treated with acetyl chloride and pyridine in order to obtain 102a (scheme XXIV). Purification by column chromatography afforded in addition to 67(a and b) only a small amount of 102b (scheme XXIV). From these results it may be concluded that any 95 formed during the course of the reaction is converted rapidly into the corresponding amine 47.

The formation of 47 may proceed via two different mechanistic pathways, namely by overreduction of 95 under the reaction conditions or by disproportionation of 95 (see for comparison eq.15) during the work-up procedure. It was impossible to deduce from the experiments which route underlies the observed product formation.

The question remains why the reduction proceeded so slowly. Although the exact mechanism of the reduction with amine-borane complexes is not known, we are inclined to contribute this failure to steric hindrance. It has been noted[83] that oximes bearing bulky substituents are reduced sluggishly. So, it is assumed that the whole amine-borane complex is involved in the hydride transfer to the protonated substrate (eq.21), thus rendering the progress of the reaction more sensitive to steric hindrance.

(eq 21)



This could be one reason for our poor results. Perhaps there is still another reason. It is well-known that boranes can form stable complexes with thioethers.[132] Since, according to the ^{1}H-NMR spectra, some of the by-products seemed to lack the sulfur-

protecting group, it is possible that displacement of the amine from the amine-borane complex by the divalent sulfur of 94 and/or 95 could be the initiating step of some side-reactions.

4.7.4 *Conclusions*

Attempts to design an alternative route to 95 have not been made since meanwhile literature reports[44] had revealed some facts pointing to a mechanism in which β-lactam formation precedes thiazolidine formation during the biosynthesis of isopenicillin N. Initial β-lactam ring formation is incompatible with the intermediacy of 36 in penicillin biosynthesis; no stepwise mechanism can be written down, exploiting both N-hydroxy functionalities of the N,N'-dihydroxy tripeptide 36.

4.8 *SYNTHESIS OF N-[δ-(L-α-AMINOADIPYL)]-N-HYDROXY-L-CYSTEINYL-D-VALINE (37)*

4.8.1 *Introduction*

In the course of our previous studies a wealth of information on penicillin biosynthesis became available from literature reports, in particular from Baldwin's group. With respect to the mechamism they gave direct evidence for initial β-lactam ring formation and they found strong indications that a radical intermediate is involved in the formation of the thiazolidine ring. The isopenicillin N synthetase seems to contain at least one cysteinyl thiol group, whose blockade inhibits the enzyme's activity : this points to the formation of a disulfide bond between the enzyme and the substrate during the conversion. These results, which are described in more detail in section 4.3, place certain restrictions on any proposed mechanism of penicillin biosynthesis. So, it became clear

that out of the three N-hydroxy Arnstein tripeptides 35-37 only 37 remained as a possible intermediate. Therefore all further attention was focused on the synthesis of this compound.

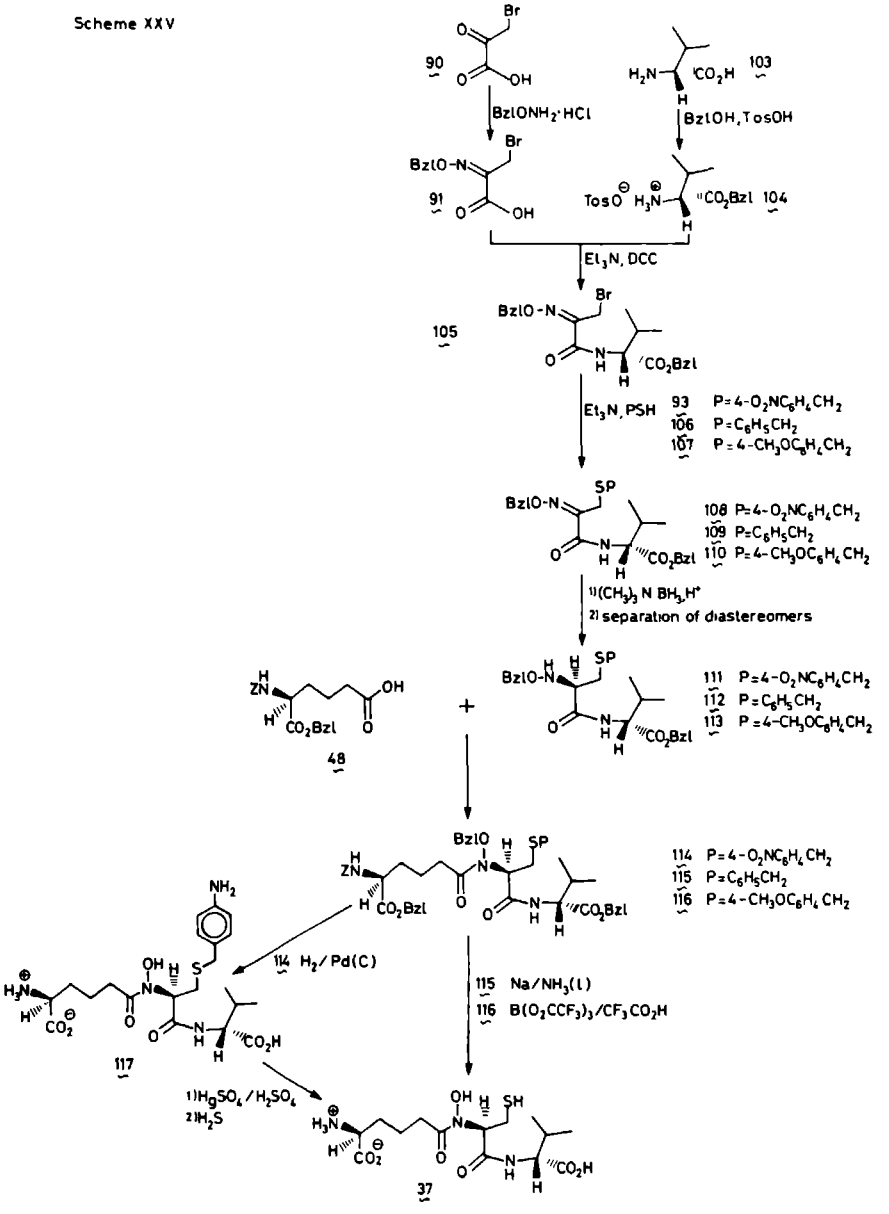
The strategy followed shows (scheme XXV) much resemblance to that used for the synthesis of 36 (see scheme XX).

This time the coupling reaction involving 91 was not expected to lead to problems with regard to the substitution of bromine (compare scheme XXI and XXII), since a simple coupling reagent like DCC would be sufficient to accomplish the reaction.

At first we intended to synthesize just one protected tripeptide, *i.e.* 114. However, since there was not much information available on the deprotection of N-hydroxy peptides, we could not be certain how the protected and deprotected compounds (114, 117 and 37) would behave under the deprotection conditions, *i.e.* in how far side-reactions would interfere with the intended reaction. Therefore we decided during the course of the investigation to readjust the protecting group strategy.

By synthesizing two additional protected tripeptides, *i.e.* 115 and 116, we hoped to avoid the risk of backing the wrong horse. Scheme XXV shows that without affecting the synthesis plan seriously various deprotection procedures could be incorporated by simply using different sulfur-protecting groups. The compounds 114-116 seemed to be readily accessible by changing the nature of the mercaptan (93, 106, 107 resp.) employed at an early stage of the synthesis (105 → 108, 109 or 110).

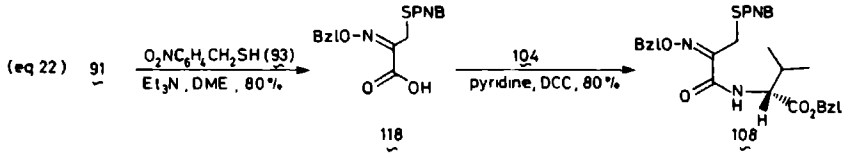
Scheme XXV


90
BzlONH2·HCl
91
103
BzlOH, TosOH
104
Et3N, DCC
105
Et3N, PSH
93 P=4-O2NC6H4CH2
106 P=C6H5CH2
107 P=4-CH3OC6H4CH2
108 P=4-O2NC6H4CH2
109 P=C6H5CH2
110 P=4-CH3OC6H4CH2
1) (CH3)3 N BH3,H+
2) separation of diastereomers
111 P=4-O2NC6H4CH2
112 P=C6H5CH2
113 P=4-CH3OC6H4CH2
48
114 P=4-O2NC6H4CH2
115 P=C6H5CH2
116 P=4-CH3OC6H4CH2
114 H2/Pd(C)
115 Na/NH3(l)
116 B(O2CCF3)3/CF3CO2H
117
1) HgSO4/H2SO4
2) H2S
37

4.8.2 *Synthesis of N-hydroxy-L-cysteinyl-D-valine derivatives 111-113*

The 4-toluenesulfonic acid salt[133] of D-valine benzyl ester 104 was obtained in 76% yield by direct esterification of D-valine 103 (scheme XXV) : this compound, an excess of benzyl alcohol and toluene were heated under reflux in the presence of 4-toluenesulfonic acid, the liberated water being removed azeotropically. Dicyclohexylcarbodiimide[134] coupling of 91, prepared according to the procedure described in section 4.7.2, and 104 gave the crude product 105 (scheme XXV) in 89% yield. Although purification of 105 is possible by column chromatography, affording the pure product in 80% yield, crude 105 was used in the next reaction. Displacement of bromine by a suitable mercaptan (scheme XXV) proceeded smoothly, affording 108, 109 or 110 in 91%, 82% and 62% yield respectively.

In one case (91 → 108) the sequence of reactions was also done in reversed order, substitution of bromine in 91 preceding coupling with the valine fragment 104 (eq. 22). This alternative synthesis of 108 gave the product in an overall yield which was somewhat lower, namely 64% versus 73%.



The next step, reduction of the oxime double bond (scheme XXV), turned out to be a more laborious job. We knew from previous work[83,92] that, probably due to competitive protonation, the presence of an amide function in the molecule slows down the reaction rate, thus causing a relatively fast decomposition of the

reducing agent under the strongly acidic conditions used. Hence, only an amine-borane complex of higher acid stability than pyridine-borane was suited for our purpose. This condition was satisfied by trimethylamine-borane.

When executing our plan it proved to be very difficult to monitor the progress of the reaction by TLC. However, after having isolated a small amount of 111 enough information was acquired to be able to optimize the reaction conditions as well as the work-up procedure. In spite of the use of a large excess of the reducing agent (14 eq.) complete conversion of the starting material was never achieved. The presence of the excess of the reducing agent and several by-products made extensive column chromatography necessary.

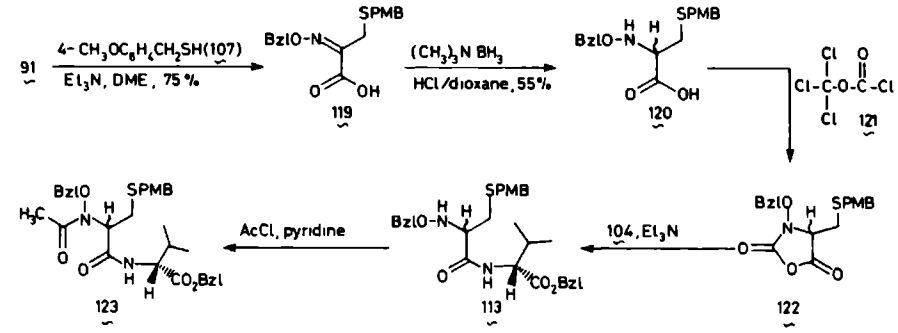
Moreover, in consequence of the introduction of a new chiral centre in the molecule the resulting mixture of diastereomers had to be separated. On TLC the mixtures of diastereomers of 111-113 gave only one spot, no matter which eluant was used. By employing careful column chromatography one pure diastereomer of 112, having an unknown configuration at the cysteine chiral centre, was obtained in 8% yield. The remaining material (38%) was a mixture of diastereomers which consisted for about 85% of the other diastereomer of 112. The separation was achieved as follows.

First, CH_2Cl_2 was used as eluant to remove the excess of the reducing agent : after 1-2h it was changed by EtOAc/hexane (35:65, v/v). In this procedure the desired compound left the column in two separate portions. Usually the first portion consisted of one diastereomer, whereas the other portion was a mixture of both diastereomers. By repeating this procedure several times with the

mixture of diastereomers, the yield of the less polar diastereomer was raised while the remaining material became strongly enriched in the other diastereomer (up to 80-90%). The diastereomers of 111 and 113 (30% and 22% yield, respectively) could not be separated in this way.

Because it is known[83,84] that the reduction of α-oximino acids proceeds much easier than that of corresponding esters or amides, we studied whether a higher yield of compound 113 could be realized when reduction preceded the coupling (scheme XXVI).

Scheme XXVI

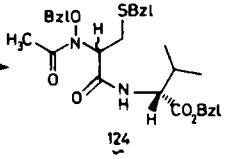


The results of the first two steps were encouraging. Substitution of 91 with the mercaptan 107 was accomplished in 75% yield. The product 119 had to be purified by column chromatography. Attempts to purify 119 or its dicyclohexylamine salt by crystallization gave much lower yields (about 26%). As anticipated the yield of the reduction (119 → 120) was better (55%) than in the former method. Besides, there was not such a large excess of reducing agent needed and the work-up procedure was less laborious. Because activation of the carboxyl group of 120 might lead to a product liable to polymerization by the presence of the weakly nucleophilic benzyloxyamino group, we used the N-carboxy anhydride

122, in which the nitrogen atom is protected, for the coupling with D-valine benzyl ester. The anhydride 122 was obtained from 120 by using trichloromethyl chloroformate 121 (diphosgene).[87a,b] This is an easy to handle liquid substitute for phosgene,[135] which is a gas at room temperature. Katakai's method,[136] which involves in situ formation of phosgene from 121 under the influence of activated charcoal, was applied in this case. After filtration the reaction mixture was concentrated in vacuo and then the residue was used immediately for the synthesis of 113.

Finally the crude reaction mixture, containing the coupling product 113, was treated with acetyl chloride and pyridine, affording the stable acetyl derivative 123. The model study of in situ acylation was carried out because there are some indications that compounds containing a free benzyloxyamino group, like *e.g.* 111-113, disproportionate (see eq.15) during laborious work-up procedures involving column chromatography.

Column chromatographic purification afforded 123 in 5% yield based on 120. Complete separation of the diastereomers did not succeed : two mixtures of diastereomers, each enriched in one of the diastereomers, were obtained. The overall yield of 123 via this route was 2% based on 91, while a similar transformation (91 → 124) according to the former route (scheme XXV, eq. 23) afforded the product in an overall yield of 12%.

(eq 23) 91 → → → 112 → 

124

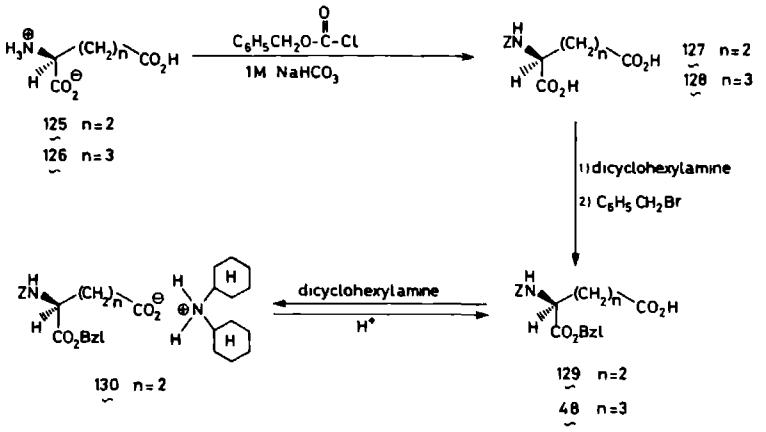
So, the route involving the intermediacy of an N-carboxy anhydride like 122 was abandoned, because ultimately the synthesis depicted in scheme XXV furnished sufficient material for further studies.

4.8.3 *Synthesis of N-benzyloxycarbonyl-L-α-aminoadipic acid α-benzyl ester*

Several methods for the synthesis of [N,C^{α}]-diprotected L-α-aminoadipic acid have been published.[98,137] For the preparation of large quantities many groups start with an efficient synthesis of L-α-aminoadipic acid, which is subsequently protected at the α-carboxyl- and amino group. Baldwin and co-workers[98b,c] developed two short routes for the synthesis of 48, which avoid the intermediacy of L-α-aminoadipic acid.

We chose a very short route, starting from the commercially available L-α-aminoadipic acid 126 (scheme XXVII).

Scheme XXVII

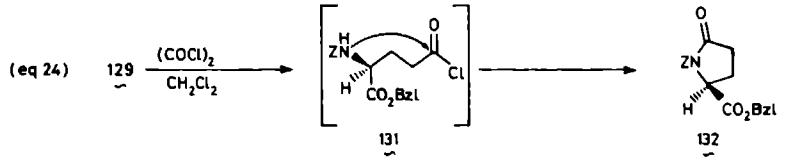


The starting material is rather expensive. For that reason we did some model experiments, using the cheaper analogue L-glutamic acid

125 as starting material (scheme XXVII : 125 → 127 → 129).

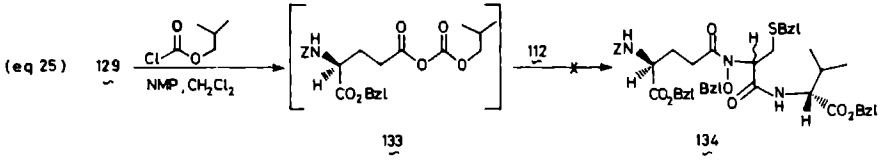
The first step, protection of the amino group of 125, was carried out under Schotten-Baumann conditions.[138] Compound 127 was obtained in 76% yield. Then, the α-carboxyl group of 127 was converted selectively into a benzyl ester (80% yield) by applying a procedure described by Nefkens.[101b] In order to secure that only the α-ester was isolated, the product was converted quantitatively into its dicyclohexylamine salt 130.[101,139] The specific rotations and melting points of the dicyclohexylamine salts of the α- and γ-ester are quite different[101,139] and can therefore be used to identify the isomers.

Subsequently, we studied the activation of the diprotected glutamic acid derivative 129. We tried to convert 129 into the corresponding acid chloride 131 (eq.24), employing very mild reaction conditions.[140]



Though the IR-spectrum revealed a strong band at 1800 cm^{-1} (C=O stretching), the compound did not behave as an activated carboxylic acid at all; *e.g.* treatment with methanol in the presence of pyridine did not yield the corresponding methyl ester. Careful inspection of the spectroscopic data revealed that 132 had been formed.[141] This pyroglutamic acid derivative[142] must have been formed by intramolecular attack of the urethane nitrogen atom on the activated carboxylic acid. Such an intramolecular cyclization is a well-known reaction of glutamic acid derivatives.[143]

In another experiment we attempted to couple compound 129 and the protected N-hydroxy peptide 112 (a mixture of diastereomers) by using the mixed anhydride method (eq. 25).



However, no reaction took place although we used optimized conditions as described by Benoiton.[128]

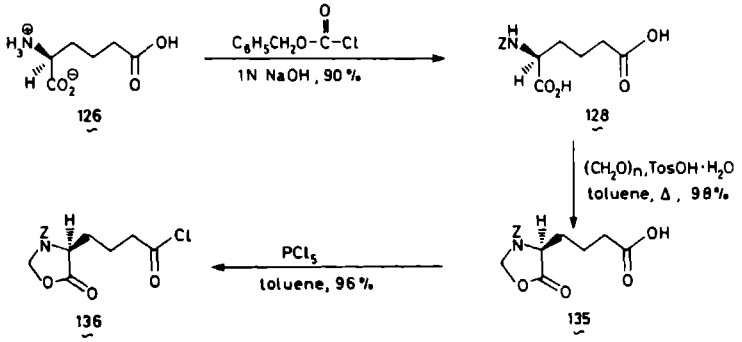
From these results we concluded that we had to face the following dilemma. To achieve an efficient coupling the carboxylic acid moiety had to be activated using a method that leads to very high activation. On the other hand ring closure as observed with the acid chloride 131 had to be avoided. We reasoned that a way out might be placing a second protecting group on the urethane nitrogen.

4.8.4 *Revised approach for the protection of L-α-aminoadipic acid*

An efficient way to accomplish a suitable protection of the urethane nitrogen atom might be ring closure involving the α-carboxyl group,[144] which would then be protected too. This approach has been used before for the protection of glutamic acid and its conversion into the γ-acid chloride.[112,144c] Scheme XXVIII shows the synthesis plan, featuring this approach.

Protection of the amino group of L-α-aminoadipic acid (126 → 128) and subsequent condensation with paraformaldehyde (128 → 135) proceeded smoothly. The product, an oil with very little impurity, was used in the next step without purification. The acid chloride 136 was obtained in nearly quantitative yield by treatment of 135 with PCl_5.[112] Its purity was at least 87% as estimated from the

Scheme XXVIII

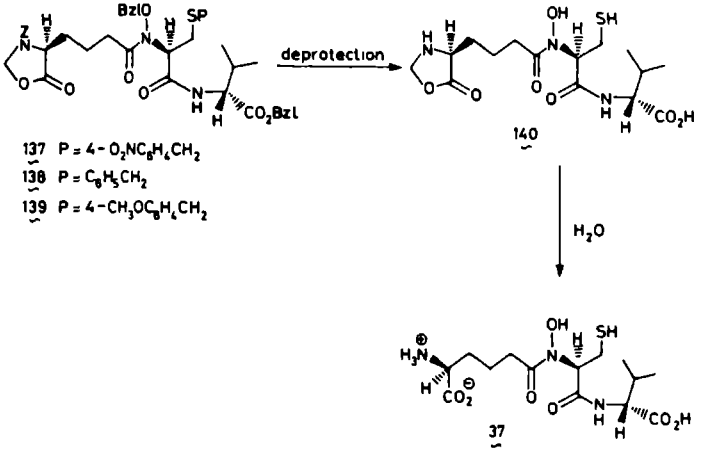


126 128 135 136

^{1}H-NMR spectrum. In contrast with the glutamic acid analogue (m.p.: 76-78°C),[144c] compound 136 was obtained as an oil. All attempts to crystallize the compound failed.

It must be emphasized that the introduction of the cyclic system was still compatible with the deprotection procedures we had in mind. We expected that after removal of the protecting groups, including the benzyloxycarbonyl-group, the remaining cyclic system (see 140, eq.26) would be sufficiently labile to be cleaved with H_2O.

(eq 26)



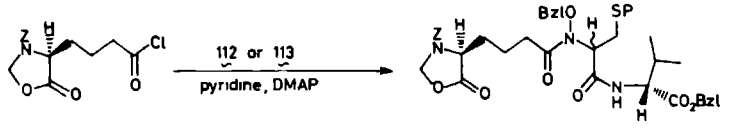
137 P = 4-$O_2NC_6H_4CH_2$
138 P = $C_6H_5CH_2$
139 P = 4-$CH_3OC_6H_4CH_2$

140 37

4.8.5 *Synthesis of N-[δ-(L-α-aminoadipyl)]-N-hydroxy-D,L-cysteinyl-D-valine derivatives 138 and 139*

Reaction of an excess (1.5-2.0 eq.) of the crude acid chloride 136 with the protected N-hydroxy dipeptides 112 and 113, in the presence of pyridine (1.5-2.0 eq.) and a catalytic amount of 4-dimethylaminopyridine, afforded the fully protected tripeptides 138 and 139 in 81% and 58% yield respectively (eq.27).

(eq 27)



136

138 P = $C_6H_5CH_2$
139 P = 4-$CH_3OC_6H_4CH_2$

The reaction was carried out with the less polar diastereomer of 112 as well as with the mixture of diastereomers which was enriched (80-90%) in the more polar diastereomer. In the latter case a mixture of diastereomers of 138 was obtained : these diastereomers could be separated completely by employing the same chromatographic procedure as used for the separation of the diastereomers at the dipeptide stage (see section 4.8.2). Thus, both diastereomers of 138 were obtained in pure form.

For the coupling reaction between 136 and 113 only a mixture of diastereomers of 113 was available. After coupling and purification a small amount (9%) of the less polar diastereomer of 139 was obtained; the remainder (49%) was again a mixture of diastereomers. The absolute configuration of the cysteine chiral centre in the pure diastereomers was not established.

Compound 137 (eq.26) has not been prepared because it seemed

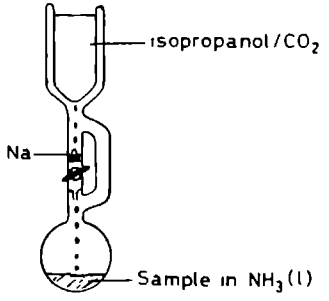
ultimately the least attractive one. Deprotection of 137 (compare scheme XXV) would involve the formation of a mercury complex of the deprotected tripeptide 37. From Prof. Baldwin[145] we learned that traces of mercury, which are difficult to remove from such a tripeptide, may inhibit the activity of the isopenicillin N synthetase during an incubation experiment. Besides, it became clear that treatment with H_2S, needed to liberate the tripeptide from its mercury complex, might cause reduction of the hydroxamic acid function to the corresponding amide.[88]

4.8.6 *Deprotection of N-[δ-(L-α-aminoadipyl)]-N-hydroxy-D,L-cysteinyl-D-valine derivatives 138 and 139*

In theory it should be possible to remove all the protecting groups from 138 and 139 in one step by making use of Na/NH_3 (l)[90,125] and $B(O_2CCF_3)_3/CF_3COOH$[85a,b,146] respectively, followed by a work-up procedure involving the use of H_2O (eq.26, compare scheme XXV).

For the reduction with $Na/NH_3(l)$ we devised a special apparatus Fig. 3.

mild reduction with $Na/NH_3(l)$

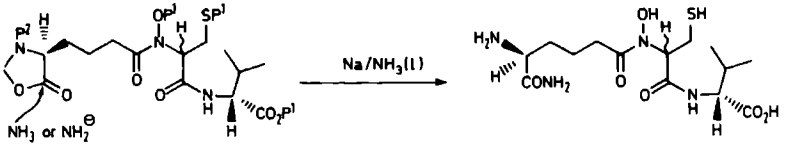


assembly, which is pictured schematically in figure 3.

In a separate vessel, charged with several pieces of freshly cut sodium metal, ammonia was condensed. Then, the liquid ammonia was distilled under argon into the reaction vessel, containing the compound to be deprotected (138). Under vigorous stirring the starting material dissolved completely in the liquid ammonia, after which the ammonia was allowed to reflux. The condensing ammonia dissolved the sodium metal, which was placed on a sintered glass disc in the dropping funnel. Addition of the reducing agent to the reaction mixture could be controlled by the stopcock. As soon as the blue colour persisted for at least one minute, the addition was stopped. This reaction can be considered as a titration with solvated electrons. The advantage of the method is that moisture is excluded and the presence of a large excess of reducing agent in the reaction mixture is avoided.

Compound 138 was deprotected in this way (eq.26). The mass spectrum of the crude product indicated that in addition to the desired compound 37 (M=379) another product had been formed, having a molecular weight (M=378) which was one unit lower than that of 37. The formation of compound 141 (eq.28), as a result of nucleophilic attack by NH_3 or NH_2^- on the ring system, is consistent with this observation.

(eq 28)

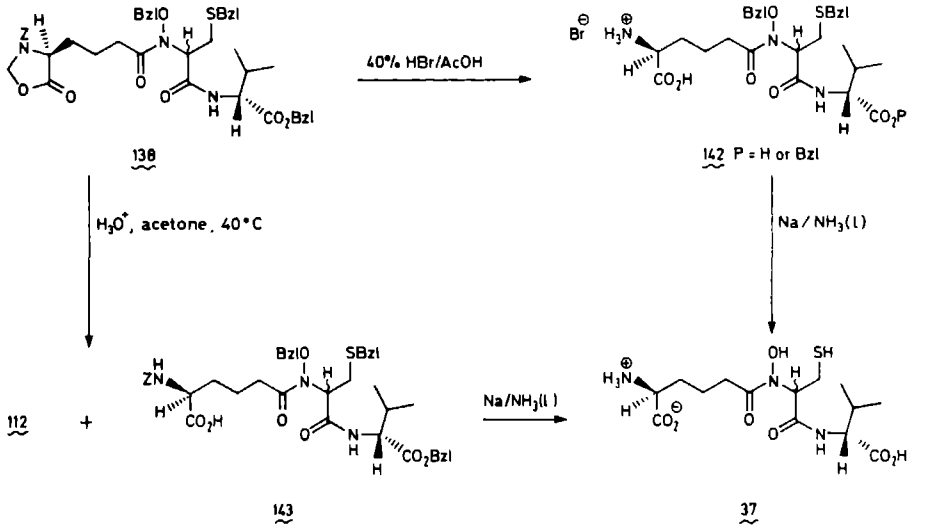


P^1 = H or $C_6H_5CH_2$
P^2 = H or $C_6H_5CH_2OC(O)$

141

It was clear that, in order to circumvent this side reaction, the deprotection procedure had to be modified in such a way that ring opening preceded sodium-liquid ammonia treatment. Scheme XXIX summarizes our attempts to achieve this.

Scheme XXIX

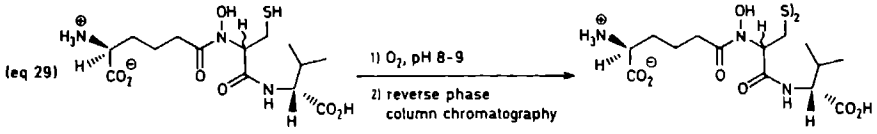


One approach involved treatment of compound 138 with HBr/AcOH.[144b] It cannot be excluded that under these conditions the benzyl ester is also converted partially into the carboxylic acid. The crude product (142) was not characterized but subjected to sodium-liquid ammonia deprotection without further purification.

In another attempt we tried to split up the cyclic system by simple hydrolysis. From literature reports[144] we knew that this could be accomplished easily under alkaline conditions. However, under such conditions the starting material would be prone to epimerization and β-elimination. Therefore we had to employ acidic

conditions, for which we selected H_3O^+/acetone. The crude product which was used as such in the sodium-liquid ammonia reaction, consisted of three compounds : besides the desired product 143 small amounts of starting material 138 and of 112 were present, the latter obviously arising from hydrolysis of the oxidized amide bond. It was gratifying to conclude that both methods yielded a crude product that, according to mass spectroscopy, contained the desired product 37.

The deprotected material was purified[147] according to a procedure developed by Baldwin's group for the purification of the Arnstein tripeptide and several analogous compounds. Following this procedure a basic aqueous solution (pH 8-9) of the crude product was oxygenated and then subjected to reverse phase column chromatography (eq.29).



37 144

It appeared that a large number of by-products had been formed during the deprotection step, probably by β-elimination and epimerization at one or more of the chiral centres. Furthermore, is should be realized that the oxidation step increases the number of products, because several combinations between compounds containing a free thiol group are possible. This is also the reason why a mixture of diastereomers is less suited for the deprotection reaction.

It turned out that in all cases in which sodium-liquid ammonia

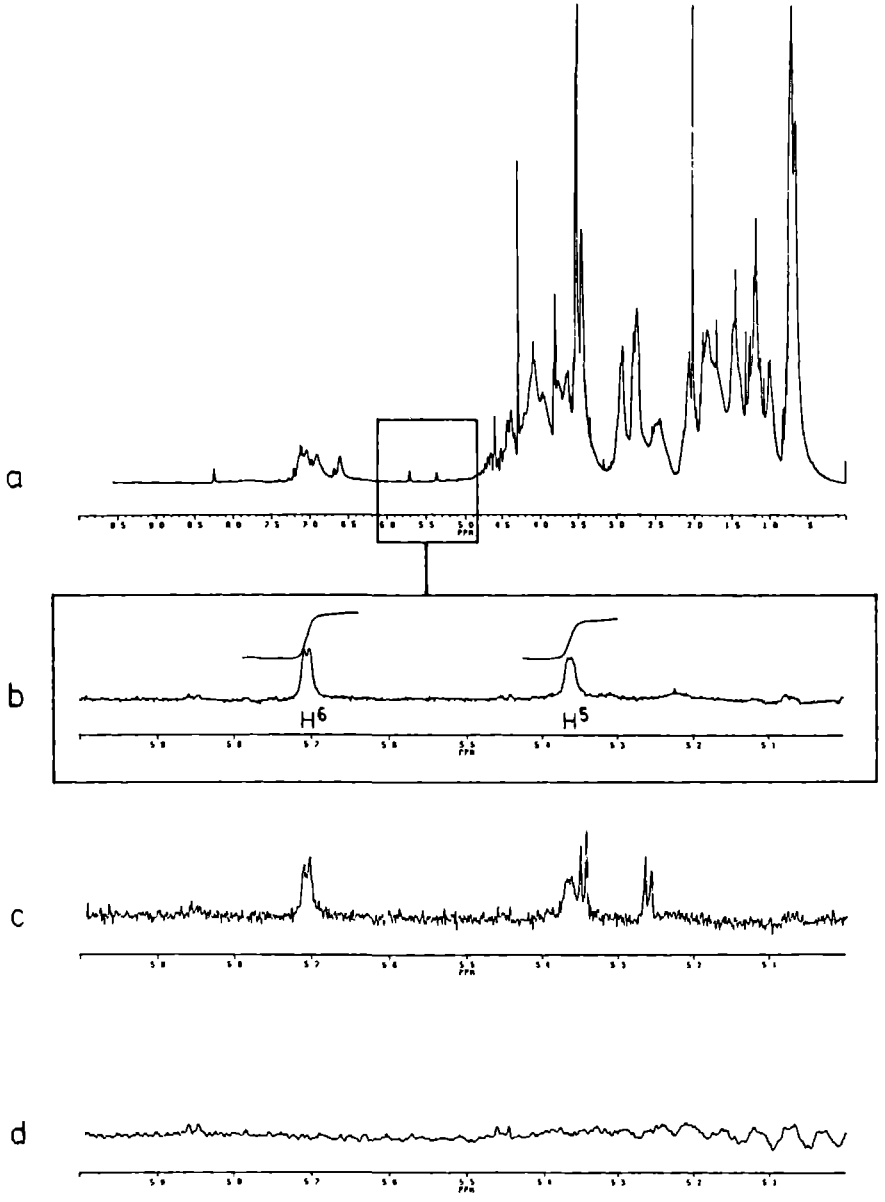
had been applied, the crude product was too contaminated to afford pure 144. Deprotection of the only available pure diastereomer of compound 139 (eq.26) with $B(O_2CCF_3)_3/CF_3COOH$ according to a literature procedure,[85b,146] followed by the same purification procedure as described in eq.29, afforded finally the pure N-hydroxy Arnstein tripeptide 37 (as its disulfide 144) in 2% yield.

Removal of the various side products by column chromatography was a time consuming job, in particular because the product, contrary to expectation, migrated much slower than the dimer of the 'normal' Arnstein tripeptide 14. Since a lot of α-aminoadipic acid, or perhaps its cyclic amide (δ-lactam), was isolated after reverse phase column chromatography, we assume that the low yield is caused by considerable hydrolysis of the oxidized amide bond during the work-up procedure.

4.8.7 *In vitro incubation of the N-hydroxy Arnstein tripeptide 37 with isopenicillin N synthetase*

The pure diastereomer of 37, obtained after deprotection of 139 and of unknown chirality at the cysteine chiral centre, was tested as a substrate of isopenicillin N synthetase. For that purpose the dimer 144 of the N-hydroxy tripeptide was administered to a purified enzyme system, derived from homogenized cells of Cephalosporium acremonium, under standard conditions.[36,46c] The ^{1}H-NMR spectrum of the incubation mixture revealed the presence of two signals between δ 5-6 ppm (fig. 4a and 4b) : the signals of the β-lactam protons of isopenicillin N usually appear in this area. Doping the NMR-sample with a small amount of penicillin N, which cannot be distinguished from isopenicillin N by NMR-spectroscopy,[145,148] proved that the compound formed was not isopenicillin N (fig. 4c).

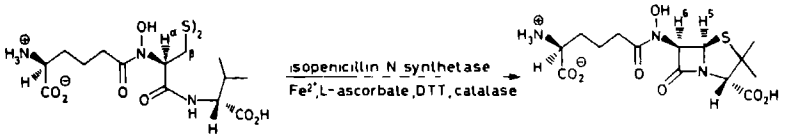
Fig.4



All the signals between δ 5 and 6 ppm disappeared after treatment of the sample with β-lactamase (fig. 4d).

In a hole-plate assay[17,149] the incubation mixture showed antibacterial activity against Staphylococcus aureus : this activity was also destroyed by the action of β-lactamase. Obviously the starting material was converted quantitatively into a new β-lactam antibiotic. As a tentative conclusion of the observations we propose that N-hydroxy isopenicillin N (145) had been formed (eq.30).

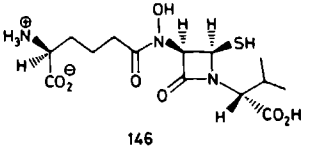
(eq 30)



144 145

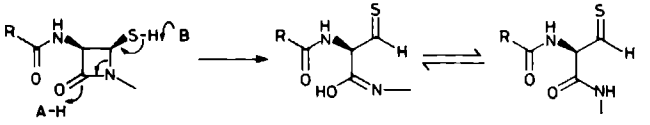
The downfield shift of the β-lactam protons H^5 and H^6, as compared with the corresponding protons of isopenicillin N (fig. 4c), can be attributed to the presence of the hydroxamic acid function. In the ^{1}H-NMR spectra of the disulfides of 37 and the Arnstein tripeptide 14 the α- and β-protons of the cysteine moiety show a comparable difference.

Structure 146 can certainly not be assigned to the novel product, since it has been shown that the comparable monocyclic



146

(eq 31)

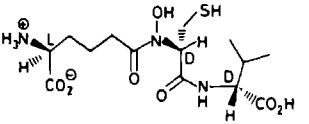


compound, lacking the hydroxyl group on the exocyclic nitrogen atom, decomposes within a few minutes under the incubation conditions (eq.31).[46]

4.8.8 *Conclusions*

The N-hydroxy cysteine derivatives 111-113, 138, 139 and 37 have been synthesized for the first time. Whereas in the synthesis of the compounds 111-113, 138 and 139 most reactions proceeded efficiently, the results of our attempts to prepare the N-hydroxy Arnstein tripeptide 37 from the fully protected tripeptides 138 and 139 were rather disappointing. We succeeded in isolating only a small amount of the desired product. Although time did not allow us to study optimization of the reaction conditions and the work-up procedures, we consider that there is still room for improvement of the deprotection procedures.

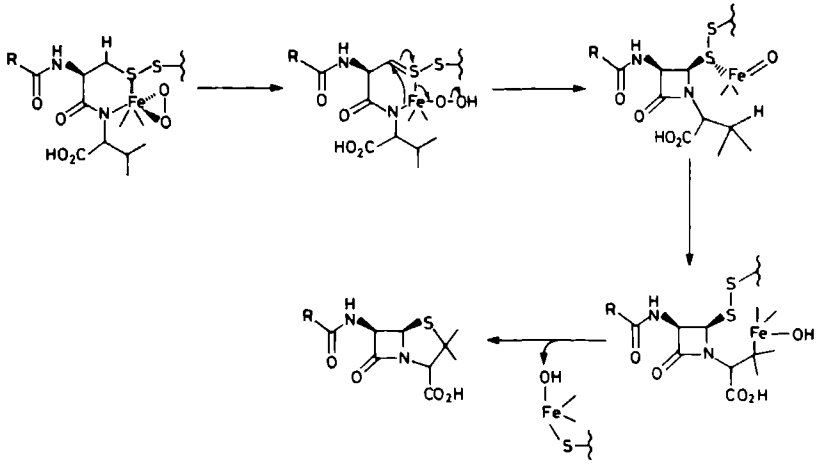
It is clear that the N-hydroxy Arnstein tripeptide 37 is not involved as an intermediate in the enzymatic conversion of the Arnstein tripeptide 14 into isopenicillin N 15. However, the incubation experiment involving 144 may be the first example of the conversion of an N-substituted Arnstein tripeptide into the corresponding penicillin. Work is in progress to isolate the product and to collect support for the proposed structure 145. Because the absolute configuration of the cysteine chiral centre in the tested compound 37 is not known, Baldwin's group is currently attempting to isolate the other diastereomer and to test it as a substrate of isopenicillin N synthetase : it is expected that this compound is the (L,D,D)-N-hydroxy tripeptide 147, which will probably not act as a substrate for the enzyme.



147

Since the postulate underlying our investigations turned out to be incorrect, the mechanism of the formation of the β-lactam ring during penicillin biosynthesis remains still unknown. Recently, Baldwin[28] proposed a mechanism which accounts for the closure of both rings (scheme XXX). In this model a crucial role is assigned to an iron ion.[28,74,75] In view of this proposal, involving the formation of a tripeptide-iron-enzyme complex, conversion of the N-hydroxy Arnstein tripeptide 37 into an isopenicillin N analogue should be rather surprising!

Scheme XXX



Much more likely would be the inhibition of the biosynthetic conversion due to complexation of the iron ion with the hydroxamic acid moiety. In this connection it is worth noting that several

hydroxamic acids are known to be powerful active, site-specific inhibitors of a number of metalloenzymes.[150] Besides, it is astonishing that the isopenicillin N synthetase tolerates so many substantial variations of the substrate[28,66-74] (see also section 4.3.4.3).

In spite of all investigations, elucidation of the exact biochemical mode of action of this enzyme remains for the time being a real challenge.

4.9 *EXPERIMENTAL SECTION*

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Infrared spectra were measured with a Perkin Elmer Model 397 spectrophotometer. Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values (parts per million) relative to tetramethylsilane as an internal standard : deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer.

Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with an UV hand lamp, iodine vapor or Cl_2-TDM.[151] Sulfur-containing compounds gave sometimes characteristic yellow spots on TLC upon spraying with a 2% $AgNO_3$ (w/v) solution in water-ethanol (4:1, v/v) and 0.1N $K_2Cr_2O_7/CH_3COOH$ (1:1, v/v).[152] Hydroxamic acids were detected (red spots) by spraying with a 3% $FeCl_3$ (w/v) solution in concentrated HCl/MeOH (4:96, v/v).

For column chromatography Merck silica gel H (type 60) was used. The Miniprep LC (Jobin Yvon) was used for preparative HPLC. Solvent

systems used were as follows : system A, CH_2Cl_2/n-hexane (1:1, v/v); system B, CH_2Cl_2; system C, EtOAc/n-hexane (35:65, v/v); system D, $CHCl_3$/MeOH/HOAc (17:2:1, v/v/v); system E, EtOAc/n-hexane (1:2.5, v/v); system F, toluene/ethyl formate/formic acid (10:7:3, v/v/v); system G, MeOH/CH_2Cl_2 (0.5:99.5, v/v); system H, MeOH/CH_2Cl_2 (1:99, v/v); system I, 1.2-dichloroethane/n-hexane (2.5:1, v/v); system J, EtOAc/n-hexane (1:3, v/v); system K, EtOAc/n-hexane (1:1, v/v); system L, CH_2Cl_2/n-hexane (9:1, v/v); system M, EtOAc/n-hexane (4:1, v/v); system N, CH_2Cl_2/n-hexane (3:1, v/v); system O, CH_2Cl_2/MeOH/HOAc (96.75:2.5:0.75, v/v/v); system P EtOAc/n-hexane (1:4, v/v).

S-4-Nitrobenzyl-L-cysteine 53

Compound 53 was prepared according to a slightly modified literature procedure.[99a] L-Cysteine 51 (24.2 g, 0.2 mol) was dissolved in 1N NaOH (400 ml, 0.4 mol). The solution was stirred vigorously, and 4-nitrobenzyl chloride 52 (34.3 g, 0.2 mol) dissolved in dioxane (300 ml) was added dropwise at 0°C during 30 minutes. The reaction mixture was stirred at room temperature for 16h. The resulting alkaline solution was washed twice with ether (200 ml) and acidified with concentrated HCl to pH 4.5. S-4-Nitrobenzyl-L-cysteine precipitated immediately as its hemihydrate. The product was recrystallized two times from hot water (2000 ml). Yield : 39.7 g (75%); m.p. : 193-195°C (dec.), (lit. : 233-234°C, monohydrate[99a]; 172.5-174°C, anhydrous compound[99b]); ^{1}H-NMR (D2O, TFA; t-BuOH) : δ 8.00 and 7.51 (AA'BB', 4H, C_6H_4), 4.35 (t, 1H $C\underline{H}CH_2$), 3.93 (s, 2H, $SC\underline{H}_2C_6H_4$), 3.12 (d, 2H, $CHC\underline{H}_2S$); anal. calcd. for $C_{10}H_{12}N_2O_4S \cdot 0.5H_2O$: C, 45.28; H, 4.94; N, 10.56, found : C, 45.11; H, 4.86; N, 10.53.

Benzyloxycarbonyloxyphtalimide 54

Compound 54 was prepared by using a slightly modified version of a published procedure.[100] Triethylamine (20.37 g, 0.2 mol) was added to a cooled (0°C) and stirred solution of N-hydroxyphtalimide (32.6 g, 0.2 mol) in 200 ml of DMF. The resulting, red solution was treated at 0°C with 36 ml of benzylchloroformate (purity ± 80%; 0.2 mol). After completion of the reaction, indicated by the disappearance of the red colour, the reaction mixture was filtered to remove $Et_3N.HCl$. The filtrate was poured into 300 ml of H_2O. The precipitate was collected, washed with water, and dried. Crystallization from 2-propanol afforded 54 in 91% yield; m.p. : 99-101°C; anal. calcd. for $C_{16}H_{11}NO_5$: C, 64.65; H, 3.73; N, 4.71, found : C, 64.59; H, 3.77; N, 4.72.

N-Benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteine 44

To a vigorously stirred suspension of compound 53 (hemihydrate, 13.2 g, 50 mmol) in 250 ml of CH_2Cl_2 were added successively 54 (14.85 g, 50 mmol) and Et_3N (10.11 g, 100 mmol). After stirring for 16h at room temperature 100 ml of 1N HCl was added to the clear solution and the resulting two-phase system was stirred vigorously for a few minutes. The layers were separated, and the organic layer was washed with 0.1N HCl (100 ml) and H_2O (3x80 ml), dried over Na_2SO_4, and finally concentrated in vacuo to leave crude 44 (20.55 g) as an oil.

Purification of 44 via its dicyclohexylamine salt 55[101]

Crude 44 was dissolved in 25 ml of dry EtOAc. After filtration of this solution freshly distilled dicyclohexylamine (9.05 g, 50 mmol) was added dropwise at 0°C. The salt 55 precipitated within a

few minutes. It was isolated by filtration and subsequently recrystallized twice from 2-propanol to afford pure 55 in 80% yield.

The free carboxylic acid 44 could be obtained quantitatively from its dicyclohexylamine salt 55 by washing a solution of 55 (22.9 g, 40 mmol) in 500 ml of CH_2Cl_2 several times with 0.1N HCl (200 ml). Subsequently the organic layer was washed with H_2O, dried over Na_2SO_4 and concentrated to dryness in vacuo to afford pure 44 as an oil. The compound was homogeneous on TLC (solvent system F) : Rf 0.28; ^{1}H-NMR : δ 8.08 and 7.40 (AA'BB', 4H, C_6H_4), 7.30 (s, 5H, C_6H_5), 5.91 (br.d., 1H, NH), 5.12 (s, 2H, OCH_2), 4.84-4.36 (m, 1H, $CHCO_2H$), 3.76 (s, 2H, $SCH_2C_6H_4$), 2.90 (d, 2H, $CHCH_2S$).

The preparation of the compounds 45 and 57-59 is described in the experimental section of chapter V.

N-Benzyloxy-D,L-valine benzyl ester hydrochloride 60

N-Benzyloxy-D,L-valine benzyl ester 45 was dissolved in a minimum amount of dry ether. Subsequently dry HCl-gas was passed through the cooled (0°C) solution. After precipitation of the salt 60, the HCl flow was continued for a few minutes. Then the product was collected by filtration, washed with dry ether and dried in vacuo over P_2O_5. Recrystallization from MeOH/ether afforded pure 60 in 91% yield; m.p. : 36-38°C; ^{1}H-NMR: δ 7.34 and 7.30 (2xs, 10H, $2xC_6H_5$), 5.54 (s, 2H, CH_2O), 5.29 (s, 2H, CH_2O), 4.00 (d, 1H, CHN), 3.04-2.29 (m, 1H, $CH(CH_3)_2$), 1.17 and 1.06 (2xd, 6H, $(CH_3)_2$).

N-[N'-Benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteinyl]-N-benzyloxy-D,L-valine benzyl ester 46

To a stirred and cooled (-78°C) solution of 44 (4.28 g, 11 mmol) in 100 ml of dry THF were added successively a solution of

$COCl_2$ (1.15 g, 11.5 mmol) in 4 ml of dry CH_2Cl_2 and tri-n-butylamine (2.03 g, 11 mmol). Stirring was continued for about 25 minutes; meanwhile the mixture was allowed to warm up to -20°C. At this temperature a solution of 60 (2.63 g, 7.5 mmol) and tri-n-butylamine (2.78 g, 15 mmol) in 75 ml of dry THF was added dropwise. Then the cooling bath was removed and the reaction mixture was stirred at room temperature for 2h. Subsequently the mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with 0.1N HCl, H_2O, and brine, and dried over Na_2SO_4. The residue, obtained after evaporation of the solvent, was purified by column chromatography (solvent system G), affording compound 46 as an oil in 25% yield (based on 60). This mixture of diastereomers was homogeneous on TLC (solvent system H): Rf 0.37; ^{1}H-NMR: δ 7.95 and 7.07 (AA'BB', 4H, C_6H_4), 7.33 (s, 15H, $3xC_6H_5$), 5.66 and 5.56 (2xd, 1H, NH), 5.29-4.75 (m, 7H, $3xCH_2O$ and CHNH), 4.68 and 4.58 (2xd, 1H, CHNO), 3.67-3.20 (6 lines, 2xAB spectrum, 2H, $SCH_2C_6H_4$), 2.77-2.22 (m, 3H, $CHCH_2S$ and $CH(CH_3)_2$), 1.11-0.84 (6 lines, 6H, $(CH_3)_2$); positive FAB mass spectrum, m/e (relative intensity): 686 ($[M+1]^+$, $C_{37}H_{40}N_3O_8S$, 1%), 314 ($[C_{19}H_{24}NO_3]^+$, Bzl0-NH_2-Val-OBzl, 3%), 91 ($[C_7H_7]^+$, 100%); IR ($CHCl_3$): 3420, 1740, 1720, 1665, 1520 and 1350 cm^{-1}.

N-[N'-Benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteinyl]-N-benzyloxy-D,L-valine benzyl ester 46, via the mixed anhydride method.

To a stirred and cooled (-15°C) solution of 44 (0.975 g, 2.5 mmol) and triethylamine (0.252 g, 2.5 mmol) in 10 ml of dry THF was added isobutyl chloroformate (0.360 g, 95%, 2.5 mmol). Stirring was continued at -15°C for 10 minutes. Subsequently, a solution of 45 (0.782 g, 2.5 mmol) in 10 ml of dry THF was added dropwise to the

suspension. The reaction mixture was stirred at -15°C for 30 minutes and at room temperature for 16h. Then the reaction mixture was filtered and concentrated in vacuo. Purification of the residue by column chromatography (solvent system G) afforded 46 in 1% yield and *N-benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteinyl-D,L-valine benzyl ester* 69 in 15% yield. Product 69, which might be a mixture of diastereomers, was homogeneous on TLC (solvent system H): Rf 0.28; ^{1}H-NMR (60 MHz): δ 8.04 and 7.37 (AA'BB', 4H, C_6H_4), 7.26 (s, 10H, $2xC_6H_5$), 6.93 (d, 1H, $N\underline{H}CHCO_2$), 5.90 (d, 1H, $N\underline{H}CHCH_2S$), 5.10 (br.s., 4H, $2xCH_2O$), 4.73-4.16 (m, 2H, $C\underline{H}CH_2S$ and $CHCO_2$), 3.76 (s, 2H, $SC\underline{H}_2C_6H_4$), 2.99-2.62 (m, 2H, $CHC\underline{H}_2S$), 2.45-1.79 (m, 1H, $C\underline{H}(CH_3)_2$), 0.93 and 0.82 (2xd, 6H, $(CH_3)_2$).

N-Benzyloxycarbonyl-S-(4-nitrobenzyl)-L-cysteinyl-D-valine benzyl ester 69

To a stirred and cooled (-45°C) solution of 44 (1.166 g, 3 mmol) and triethylamine (0.303 g, 3 mmol) in 10 ml of dry THF was added isobutyl chloroformate (0.431 g, 95%, 3 mmol). Stirring was continued for 10 minutes, while the temperature was allowed to rise to -15°C. At this temperature a solution of D-valine benzyl ester 4-toluenesulfonic acid salt 104 (70.TosOH, 1.137 g, 3 mmol) and triethylamine (0.303 g, 3 mmol) in 10 ml of dry THF was added dropwise. The reaction mixture was stirred at -15°C for 30 minutes and at room temperature for 16h. After removal of the precipitate by filtration, the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc, washed with H_2O and brine, and dried over Na_2SO_4. Evaporation of the solvent in vacuo, followed by purification of the residue by column chromatography (solvent system B) afforded pure 69 as a white solid in 29% yield: Rf 0.28 (solvent system H). The 60 MHz ^{1}H-NMR spectra of compound 69 and the product

obtained according to the preceding procedure (from 44 and 45) were almost identical; ^{1}H-NMR (90 MHz): δ 8.10 and 7.44 (AA'BB', 4H, C_6H_4), 7.30 (s, 10H $2xC_6H_5$), 6.70 (d, 1H, J=8.8Hz, $N\underline{H}CHCO_2$), 5.65 (d, 1H, J= 7.5 Hz, $N\underline{H}CHCH_2S$), 5.18 and 5.10 (AB spectrum, 2H, J_{AB} = 11.9 Hz, CH_2O), 5.11 (s, 2H, CH_2O), 4.55 (dd, 1H, J= 8.8 Hz resp. 4.5 Hz, $CHCO_2$), 4.46-4.23 (6 lines, 1H, $C\underline{H}CH_2S$), 3.76 (s, 2H, $SC\underline{H}_2C_6H_4$), 2.86 and 2.73 (AB part of ABX spectrum, 2H, J_{AX} = 5.3 Hz, J_{BX} = 7.0 Hz, J_{AB} = 14.0 Hz, $CHC\underline{H}_2S$), 2.46-1.87 (m, 1H, $C\underline{H}(CH_3)_2$), 0.85 (t, 6H, $(CH_3)_2$); chemical-ionization mass spectrum, m/e (relative intensity) : 580 ($[M+1]^+$, $C_{30}H_{34}N_3O_7S$, 1%), 535 ($[M-CO_2]^+$, 1%), 473 ($[M-C_7H_6O]^+$, 1%), 445 ($[M-C_8H_6O_2]^+$, 3%), 401 ($[M-C_9H_6O_4]^+$, 5%), 303 (5%), 257 (14%), 170 ($[C_7H_8NO_2S]^+$, 46%), 136 ($[C_7H_6NO_2]^+$, 10%), 106 ($[C_7H_6O]^+$, 22%), 91 ($[C_7H_7]^+$, 100%); anal. calcd. for $C_{30}H_{33}N_3O_7S$: C, 62.16; H, 5.74; N, 7.25, found : C, 62.13; H, 5.83; N, 7.21.

N-Carboxy-S-(4-nitrobenzyl)-L-cysteine anhydride 66

All reactions involving N-carboxy anhydrides were carried out in a nitrogen atmosphere. THF (30 ml) and finely ground 53 (2.65 g, 10 mmol) were charged to a three-necked, round-bottomed flask, fitted with a reflux condenser. The equipment had been flushed with dry nitrogen for 0.5h before use. Phosgene addition was begun at a moderate rate and gentle external heating was applied to bring the stirred reaction mixture to 45-50°C. Phosgene addition and stirring were continued at 45-50°C to give a clear solution, and for a further 15 min. to complete the reaction. When phosgene addition and heating had been stopped, nitrogen was bubbled through the solution to remove some of the excess phosgene (30-60 min.). Finally the mixture was filtered and evaporated to dryness in

vacuo, using a water bath at a temperature not exceeding 35°C. The greyish solid residue which was obtained in nearly quantitative yield could not be recrystallized because of decomposition; ^{1}H-NMR (d6-DMSO): δ 9.08 (s, 1H, NH), 8.13 and 7.53 (AA'BB', 4H, C_6H_4), 4.75 (t, 1H, CHN), 3.95 (s, 2H, $SCH_2C_6H_4$), 2.90 (d, 2H, $CHCH_2S$); IR(KBr): 3330 cm^{-1} (NH), 1840 and 1785 cm^{-1} (C=O).

N-(2-Nitrophenylsulfenyl)-N-carboxy-S-(4-nitrobenzyl)-L-cysteine anhydride 77

Crude 66 (2.82 g, 10 mmol) was dissolved in 30 ml of dry THF and the solution was cooled to 0°C. Then o-nitrophenylsulfenyl chloride (1.9 g, 10 mmol) was added to the stirred solution, followed by a solution of Et_3N (1.01 g, 10 mmol) in 10 ml of dry THF, which was added dropwise. The reaction mixture was stirred at 0°C for 30 minutes, after which the precipitate was removed by filtration. The filtrate was concentrated in vacuo at 35°C. Attempts to crystallize the product failed. The freshly prepared oil was used in the next reaction without further purification.

Attempted synthesis of N-[N'-(2-nitrophenylsulfenyl)-S-(4-nitrobenzyl)-L-cysteinyl]-N-benzyloxy-D,L-valine benzyl ester 78.

To a stirred solution of 60 (1.74 g, 5 mmol) in 10 ml of dry THF were added successively a solution of Et_3N (0.50 g, 5 mmol) in 10 ml of dry THF and a solution of crude 77 (4.3 g, 10 mmol) in 40 ml of dry THF. The reaction mixture was stirred at room temperature for five days. Then the precipitate was removed by filtration and the filtrate was concentrated in vacuo. The residue was dissolved in 150 ml of CH_2Cl_2, washed with a 5% aqueous citric acid solution (2x50 ml), 5% aqueous $NaHCO_3$ (50 ml) and H_2O (2x50 ml), and dried

over Na_2SO_4. The organic solvent was evaporated in vacuo to afford an oily residue (3.0 g), which was subjected to column chromatography (solvent system B). Two of the fractions obtained were further purified by preparative TLC on silica using solvent system H as eluant to afford a trace of *78* and a few mg of *79*, both as a mixture of diastereomers. Exact yields were not determined.

Compound *78*: Rf 0.30 (solvent system B); ^{1}H-NMR: δ 8.39 -7.80 and 7.67-6.84 (2xm, 18H, 2xC_6H_4 and 2xC_6H_5), 5.16-4.43 (m, 5H, 2xCH_2O and CHNO), 4.10-3.61 (m, 1H, CHNS), 3.50-3.22 (m, 3H, NH and S$CH_2C_6H_4$), 3.08-2.23 (m, 3H, $CHCH_2S$ and $CH(CH_3)_2$), 1.02 and 0.86 (2xd, 6H, $(CH_3)_2$).

Compound *79*: Rf 0.27 (solvent system B); ^{1}H-NMR: δ 8.32-7.16 (m, 13H, 2xC_6H_4 and C_6H_5), 6.86 (d, 1H, NHCO), 5.17 (s, 2H, CH_2O), 4.60 (dd, 1H, $CHCO_2$), 3.77 and 3.64 (2xs, 3H, NHS and S$CH_2C_6H_4$), 3.42 (5 lines, 1H, CHNS), 2.86 (d, 2H, $CHCH_2S$), 2.44-1.93 (m, 1H, $CH(CH_3)_2$), 0.89 (4 lines, 6H, $(CH_3)_2$).

N-(2-Nitrophenylsulfenyl)-S-(4-nitrobenzyl)-L-cysteinyl-D-valine benzyl ester 79

To stirred suspension of D-valine benzyl ester 4-toluenesulfonic acid salt 104 (1.78 g, 4.7 mmol) in 30 ml of dry THF were added successively Et_3N (0.474 g, 4.7 mmol) and a solution of crude *77* (2.15 g, 4.95 mmol) in 20 ml of dry THF. The clear solution was stirred at room temperature for one day. After filtration and evaporation of the solvent in vacuo, the resulting residue was dissolved in EtOAc, washed with a 5% aqueous citric acid solution (3x80 ml), a 5% aqueous $NaHCO_3$ solution (3x100 ml) and brine (100 ml), and dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded a waxy solid (1.7 g), which was purified by column

chromatography (solvent system B). Pure 79 was obtained as a yellow solid in 27% yield: Rf 0.27 (solvent system B); ^{1}H-NMR: δ 8.31-7.16 (m, 13H, 2xC_6H_4 and C_6H_5), 6.89 (d, 1H, J = 9.0 Hz, NHCO), 5.16 (AB spectrum, 2H, CH_2O), 4.61 (dd, 1H, J = 9.0 and 4.5 Hz, $CHCO_2$), 3.83 and 3.78 (2xs, 3H, NHS and $SCH_2C_6H_4$), 3.47 (q, 1H, J=6.0 Hz, $CHCH_2S$), 2.86 (d, 2H, J=6.0 Hz, $CHCH_2S$), 2.41-1.97 (m, 1H, $CH(CH_3)_2$), 0.88 (t, 6H, $(CH_3)_2$); chemical-ionization mass spectrum, m/e (relative intensity): 599 ($[M+1]^+$, $C_{28}H_{31}N_4O_7S_2$, 0.6%), 536 ($[M-NO_3]^+$, 0.9%), 446 ($[C_{22}H_{28}N_3O_5S]^+$, 10%), 430 ($[M-C_7H_6NO_2S]^+$, 3%), 303 ($[C_9H_9N_3O_5S_2]^+$ or $[C_{10}H_{11}N_2O_5S_2]^+$, 7%), 257 ($[C_9H_9N_2O_3S_2]^+$, 12%), 167 ($[C_7H_5NO_2S]^+$, 5%), 154 ($[C_6H_4NO_2S]^+$, 100%), 138 ($[C_7H_8NO_2]^+$, 35%), 108 ($[C_7H_8O]^+$, 13%), 91 ($[C_7H_7]^+$, 76%); anal. calcd. for $C_{28}H_{30}N_4O_7S_2$: C, 56.17; H, 5.05; N, 9.36, found: C, 55.62; H, 5.05; N, 9.20.

N-(4-Methoxybenzylidene)-D-valine benzyl ester 83

D-Valine benzyl ester 4-toluenesulfonic acid salt 104 (1.89 g, 5 mmol) was dissolved in CH_2Cl_2 and treated with triethylamine (0.505 g, 5 mmol). The resulting solution was washed thrice with H_2O, dried over Na_2SO_4, and concentrated in vacuo to afford D-valine benzyl ester 70. The free amine was dissolved in 6 ml of dry CH_2Cl_2. The solution was added to a mixture of 4-methoxybenzaldehyde (0.63 g, 4.6 mmol) and activated 4A molecular sieves (± 2 g) in 6 ml of dry CH_2Cl_2. The mixture was stirred for 16h at room temperature, filtered and concentrated in vacuo to afford compound 83 as an oil in quantitative yield; ^{1}H-NMR: δ 8.05 (s, 1H, CH=N), 7.63 and 6.81 (AA'BB', 4H, C_6H_4), 7.21 (s, 5H, C_6H_5), 5.13 (s, 2H, OCH_2), 3.74 (s, 3H, OCH_3), 3.61 (d, 1H, $CHCO_2$), 2.70-1.90 (m, 1H, $CH(CH_3)_2$), 0.93 (d, 6H, $(CH_3)_2$).

3-Bromo-2-benzyloximino-propanoic acid 91

A suspension of benzylhydroxylamine hydrochloride (Janssen Chimica, 16 g, 100 mmol) in 150 ml of H_2O was added at room temperature to a vigorously stirred solution of bromopyruvic acid 90 (Fluka AG, 16.7 g, 100 mmol) in 100 ml of H_2O. The suspension was stirred at room temperature for 2h. The crystalline product was collected by filtration and washed twice with 0.05N HCl (50 ml) and H_2O (50 ml). The compound was recrystallized from MeOH-H_2O, and subsequently dried thoroughly in a vacuum desiccator charged with phosphorus pentoxide: 91 was obtained in 88% yield. M.p.: 147-148°C; ^{1}H-NMR: δ 7.37 (s, 5H, C_6H_5), 5.40 (s, 2H, CH_2O), 4.20 (s, 2H, CH_2Br); exact mass calcd. for $C_{10}H_{11}BrNO_3$ (M^++1), m/e 271.9922, found: 271.9918; chemical-ionization mass spectrum, m/e (relative intensity): 274/272 ($[M+1]^+$, 7%), 256/254 ($M-OH]^+$, 4%), 192 ($[M-Br]^+$, 25%), 107 ($[C_7H_7O]^+$, 11%), 91 ($[C_7H_7]^+$, 100%); anal. calcd. for $C_{10}H_{10}BrNO_3$: C, 44.14; H, 3.70: N, 5.15, found: C, 44.40; H, 3.74; N. 5.10.

4-Nitrophenyl (3-bromo-2-benzyloximino)propanoate 97

DCC (1.35 g, 6.5 mmol) was added all at once to a stirred and cooled (0°C) solution of 91 (1.72 g, 6.3 mmol) and 4-nitrophenol (0.96 g, 6.9 mmol) in 50 ml of EtOAc. The reaction mixture was stirred at 0°C for 30 minutes and at room temperature for 16h. Dicyclohexylurea was removed by filtration and the solvent was evaporated in vacuo. The residue was recrystallized from 2-propanol, affording 97 as a white solid in 73% yield. The compound was homogeneous on TLC (solvent system B): Rf 0.68. M.p.: 93-95°C; ^{1}H-NMR: δ 8.18 and 7.30 (AA'BB', 4H, C_6H_4), 7.37 (s, 5H, C_6H_5), 5.43 (s, 2H, OCH_2), 4.27 (s, 2H, $BrCH_2$).

N-(3-Chloro-2-benzyloximino-propanoyl)-N-benzyloxy-D,L-valine benzyl ester 98

Isobutyl chloroformate (0.29 g, 95%, 2.0 mmol) was added to a stirred and cooled (-5°C) solution of 91 (0.57 g, 2.1 mmol) and N-methylpiperidine (0.20 g, 2.0 mmol) in 40 ml of dry CH_2Cl_2. After 10 minutes a cold solution of 60 (=45.HCl, 0.70 g, 2.0 mmol) and N-methylpiperidine (0.20 g, 2 mmol) in a few ml of dry CH_2Cl_2 was added dropwise to the cooled reaction mixture. Stirring was continued for 1h at -5°C and for 16h at room temperature. After being washed with 0.1M aqueous citric acid, the reaction mixture was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (solvent system I), affording the chloro-compound 98 in 45% yield. The product was homogeneous on TLC (solvent system I): Rf 0.62; ^{1}H-NMR: δ 7.27 (m, 15H, 3x C_6H_5), 5.18 (s, 2H, OCH_2), 5.13 (2, 2H, OCH_2), 4.93 (s, 2H, $C(O)NOCH_2$), 4.63 (d, 1H, $CHCO_2$), 4.36 (AB spectrum, 2H, CH_2Cl), 2.79-2.27 (m, 1H, CH $(CH_3)_2$), 1.02 and 0.93 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{32}ClN_2O_5$ (M^++1), m/e 523.200, found 523.198; chemical-ionization mass spectrum, m/e (relative intensity): 613/615 ($[M+C_7H_7]^+$, 9%), 523/525 ($[M+1]^+$, 32%), 487 ($[M-Cl]^+$, 5%), 314 ($[C_{19}H_{24}NO_3]^+$, 3%), 181 ($[C_{14}H_{13}]^+$, 25%), 107 ($[C_7H_7O]^+$, 14%), 91 ($[C_7H_7]^+$, 15%), 77 $[C_6H_5]^+$, 100%).

3-Bromo-2-benzyloximino-propanoyl bromide 99

PBr_3 (61.3 g, 226 mmol) was added dropwise to a suspension of 91 (9.96 g, 36.6 mmol) in 23 ml of dry ether at room temperature. Subsequently, the reaction mixture was heated under reflux for 3 days. After cooling to room temperature the brown solution was decanted and the residual phosphorous acid was washed twice with

dry ether. The combined ethereal solutions were concentrated in vacuo after which the excess PBr_3 (b.p. 53°C, 8 mmHg) was removed by distillation under diminished pressure, leaving crude 99 (10.7 g, ≤ 87%) in the distillation flask. The crude product, probably a mixture of the E- and Z-isomer, was used in the next reaction without further purification; ^{1}H-NMR (CCl_4): δ 7.25 (s, 5H, C_6H_5), 5.40 and 5.27 (2xs, 2H, OCH_2), 3.97 and 1.98 (2xs, 2H, CH_2Br); IR (CCl_4) : 1760, 1740, 1580, 1025, 880 and 700 cm^{-1}.

N-(3-Bromo-2-benzyloximino-propanoyl)-N-benzyloxy-D,L-valine benzyl ester 92

The free amine of 60 was obtained in quantitative yield by washing a solution of 60 (1.36 g, 3.9 mmol) in 35 ml of CH_2Cl_2 with H_2O (2x 20 ml), drying the organic layer over Na_2SO_4, and evaporating the solvent in vacuo. The residue (45, 1.22 g, 3.9 mmol) was dissolved in 5 ml of dry CH_2Cl_2. To the cooled (0°C) solution were added successively a solution of the crude acid bromide 99 (1.51 g, ≤ 4.5 mmol) in 5 ml of dry CH_2Cl_2 and a solution of pyridine (0.308 g, 3.9 mmol) in 2 ml of dry CH_2Cl_2. After completion of the addition the reaction mixture was stirred at room temperature for 5h. The precipitate was removed by filtration, and the filtrate was diluted with CH_2Cl_2 to a total volume of 35 ml. The solution was washed with an 0.1M aqueous citric acid solution (2x 25 ml) and H_2O (25 ml), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (solvent system I) to afford pure 92 as an oil in 54% yield. The product was homogeneous on TLC (solvent system I): Rf 0.62; ^{1}H-NMR: δ 7.23 (s, 15H, 3xC_6H_5), 5.16 (s, 2H, OCH_2), 5.10 (s, 2H, OCH_2), 4.92 (s, 2H, $C(O)NOCH_2$), 4.60 (d, 1H, $CHCO_2$), 4.16 (s, 2H, CH_2Br), 2.76-2.29 (m, 1H,

$C\underline{H}(CH_3)_2$), 1.00 and 0.92 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{32}BrN_2O_5$ (M^++1), m/e 567.149, found : 567.148; chemical-ionization mass spectrum, m/e (relative intensity): 595/597 ($[M+C_2H_5]^+$, 3%), 567/569 ($[M+1]^+$, 25%), 487/489 ($[M+C_2H_5-C_7H_8O]^+$, 8%), 397 ($[M-C_7H_6Br]^+$, 15%), 181 ($[C_{14}H_{13}]^+$, 20%), 107 ($[C_7H_7O]^+$, 27%), 91 ($[C_7H_7]^+$, 100%).

4-Nitrobenzyl mercaptan (4-nitro-α-toluenethiol) 93

This preparation was carried out under a nitrogen atmosphere. Potassium thioacetate (11.4 g, 0.1 mol) was added all at once to a stirred solution of 4-nitrobenzyl chloride (17.2 g, 0.1 mol) in a mixture of EtOH (200 ml) and 1,4-dioxane (50 ml). Stirring was continued until completion of the reaction (± 2h) as monitored by TLC (solvent system J). Then the solvent was evaporated in vacuo. EtOAc was added to the residue and the resulting suspension was washed twice with water. After drying the clear organic layer over Na_2SO_4 the solvent was evaporated in vacuo. The residue was recrystallized from EtOAc/petroleum ether 60°-80° to afford *4-nitrobenzyl thioacetate 100* as a pale yellow solid in 92% yield.

The thioester *100* (21.1 g, 0.1 mol) was dissolved in 375 ml of dry MeOH under a nitrogen atmosphere. HCl was passed through the solution until completion of the reaction (± 2h) as monitored by TLC (solvent system K). Then the reaction mixture was filtered and concentrated in vacuo. The residue was recrystallized from EtOH (± 250 ml) to afford 4-nitrobenzyl mercaptan as a pale yellow solid in 87% yield; m.p.: 55-57°C (lit.[153]: 57-58°C); ^{1}H-NMR: δ 8.07 and 7.38 (AA'BB', 4H, C_6H_4), 3.79 (d, 2H, CH_2S), 1.83 (t, 1H, SH).

N-[3-(4-Nitrobenzylthio)-2-benzyloximino-propanoyl]-N-benzyloxy-D,L-valine benzyl ester 94

A solution of triethylamine (0.124 g, 1.22 mmol) in 10 ml of dry DME was added dropwise to a stirred solution of 92 (0.683 g, 1.20 mmol) and 4-nitrobenzyl mercaptan (0.216 g, 1.28 mmol) in 20 ml of dry DME at room temperature and in an argon atmosphere. Stirring was continued until completion of the reaction (± 6h) as monitored by TLC (solvent system L). Triethylamine hydrobromide was removed by filtration and the solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (50 ml), washed with 0.05N HCl (30 ml) and H_2O (30 ml). After drying over Na_2SO_4 and evaporation of the solvent in vacuo, the residue was purified by column chromatography (solvent system L). Pure 94 was obtained as a solid in 55% yield. The compound was homogeneous on TLC (solvent system L): Rf 0.57; ^{1}H-NMR: δ 7.95 and 7.18 (AA'BB', 4H, C_6H_4), 7.27 (s, 15H, 3x C_6H_5), 5.19 (s, 2H, OCH_2), 5.11 (s, 2H, OCH_2), 5.00 (AB spectrum, 2H, $C(O)NOCH_2$), 4.72 (d, 1H, $CHCO_2$), 3.60 (s, 2H, CH_2S), 3.43 (s, 2H, CH_2S), 2.75-2.35 (m, 1H, $CH(CH_3)_2$), 1.03 (d, 6H, $(CH_3)_2$); chemical-ionization mass spectrum, m/e (relative intensity): 656 ($[M+1]^+$, $C_{36}H_{38}N_3O_7S$, 4%), 550 ($[M-C_7H_5O]^+$, 5%), 170 ($[C_7H_8NO_2S]$, 5%), 106 ($[C_7H_6O]^+$, 24%), 102 (57%), 92 ($[C_7H_8]^+$, 14%), 85 (100%).

Attempted synthesis of N-[N'-benzyloxy,N'-acetyl-S-(4-nitrobenzyl)-D,L-cysteinyl]-N-benzyloxy-D,L-valine benzyl ester 102a

With intervals of several hours seven portions of pyridine.BH_3, 0.043 g (0.46 mmol) per portion, were added to a solution of 94 (0.30 g, 0.46 mmol) in a few ml of dry Et_2O, saturated with dry HCl. After stirring at room temperature for two days the reaction

was complete as monitored by TLC (solvent system L). The reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with 0.1N HCl, 1% aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue (0.185 g) was dissolved in 1 ml of dry CH_2Cl_2. To the stirred solution were added successively a solution of acetyl chloride (0.044 g, 0.56 mmol) in dry CH_2Cl_2 (2 ml) and a solution of pyridine (0.044 g, 0.56 mmol) in dry CH_2Cl_2 (2 ml). After stirring at room temperature for 16h the reaction mixture was diluted by adding 15 ml of CH_2Cl_2. Subsequently the solution was washed with 0.05N HCl (20 ml) and H_2O (20 ml), dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography (solvent systems successively E, K and M) afforded, among others, the following oily products: *102b*, 7% yield; the compound, probably a racemate, was homogeneous on TLC (solvent system M): Rf 0.82; ^{1}H-NMR: δ 7.95 and 7.07 (AA'BB', 4H, C_6H_4), 7.66-7.18 (m, 5H, C_6H_5), 7.33 (s, 5H, C_6H_5), 6.19 (d, 1H, NH), 5.55-5.28 (m, 1H, CHNH), 5.25 and 4.80 (AB spectrum, 2H, J_{AB} = 9.4 Hz, $NOCH_2$), 5.20 (s, 2H, CO_2CH_2), 4.67 (d, 1H, CHNO), 3.56 and 3.44 (AB spectrum, 2H, J_{AB} = 13.4 Hz, $C_6H_4CH_2S$), 2.75-2.29 (m, 3H, $CHCH_2S$ and $CH(CH_3)_2$), 2.07 (s, 3H, $C(O)CH_3$), 0.94 (t, 6H, $(CH_3)_2$); chemical-ionization mass spectrum, m/e (relative intensity): 594 ($[M+1]^+$, $C_{31}H_{36}N_3O_7S$, 3%), 488 ($[M-C_7H_5O]^+$, 3%), 314 ($[C_{19}H_{24}NO_3]^+$, BzlO-NH_2-Val-OBzl, 37%), 206 ($[C_{12}H_{16}NO_2]^+$, 10%), 170 ($[C_7H_8NO_2S]^+$, 16%), 138 ($[C_7H_8NO_2]^+$, 9%), 107 ($[C_7H_7O]^+$, 39%), 91 ($[C_7H_7]^+$, 91%), 41 (100%).

67a, 1% yield; the compound was homogeneous on TLC (solvent system M) : Rf 0.66; ^{1}H-NMR : 8.20 and 7.49 (AA'BB', 4H, C_6H_4),

7.36 (s, 5H, C_6H_5), 6.15 (br.s., 1H, NH), 4.99 and 4.89 (AB spectrum, 2H, J_{AB} = 10.2 Hz, OCH_2), 4.07-3.77 (m, 2H, CHNO and $C\underline{H}CH_2S$), 3.84 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.20 and 2.70 (AB part of ABX spectrum, 2H, J_{AX} = 3.3 Hz, J_{BX} = 8.8 Hz, J_{AB} = 14.0 Hz, $CHC\underline{H}_2S$), 2.66-2.23 (m, 1H, $C\underline{H}(CH_3)_2$), 0.99 (t, 6H, $(CH_3)_2$); exact mass calcd. for $C_{22}H_{26}N_3O_5S$ (M^++1), m/e 444.1593, found 444.1589; chemical-ionization mass spectrum, m/e (relative intensity) : 444 ($[M+1]^+$, 9%), 338 ($[M-C_7H_5O]^+$, 7%), 275 ($[M-C_7H_6NO_2S]^+$, 3%), 170 ($[C_7H_8NO_2S]^+$, 7%), 138 ($[C_7H_8NO_2]^+$, 4%), 107 ($[C_7H_7O]^+$, 20%), 106 ($[C_7H_6O]^+$, 30%), 91 ($[C_7H_7]^+$, 74%), 41 (100%).

67b, 6% yield; the compound was homogeneous on TLC (solvent system M) : Rf 0.55; ^{1}H-NMR : δ 8.19 and 7.48 (AA'BB', 4H, C_6H_4), 7.36 (s, 5H, C_6H_5), 6.34 (br.s., 1H, NH), 4.98 and 4.92 (AB spectrum, 2H, J_{AB} = 9.9 Hz, OCH_2), 4.10-3.86 (m, 2H, CHNO and $C\underline{H}CH_2S$), 3.82 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.22 and 2.60 (AB part of ABX spectrum, 2H, J_{AX} = 2.7 Hz, J_{BX} = 10.5 Hz, J_{AB} = 13.7 Hz, $CHC\underline{H}_2S$), 2.56-2.24 (m, 1H, $C\underline{H}(CH_3)_2$), 1.05 and 0.95 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{22}H_{26}N_3O_5S$ (M^++1), m/e 444.1593, found 444.1589; chemical-ionization mass spectrum, m/e (relative intensity) : 444 ($[M+1]^+$, 5%), 338 ($[M-C_7H_5O]^+$, 6%), 275 ($[M-C_7H_6NO_2S]^+$, 16%), 170 ($[C_7H_8NO_2S]^+$, 39%), 138 ($[C_7H_8NO_2]^+$, 11%), 107 ($[C_7H_7O]^+$, 41%), 106 ($[C_7H_6O]^+$, 45%), 91 ($[C_7H_7]^+$, 100%).

D-Valine benzyl ester 4-toluenesulfonic acid salt 104[133]

D-Valine 103 (Janssen Chimica, 11.7 g, 100 mmol) and p-toluenesulfonic acid monohydrate (22.8 g, 120 mmol) were pulverized and added to a mixture of 75 ml of benzyl alcohol and 100 ml of toluene in a 500 ml round-bottomed flask. The suspension was heated under

reflux to remove the water azeotropically. The water was trapped with the aid of a Dean-Stark apparatus. A clear solution was obtained soon after reflux began. After 3-4h of reflux the reaction mixture was allowed to cool to room temperature. The desired compound precipitated and was isolated by filtration with suction. The crude product was washed with toluene and cold ether, and recrystallized from MeOH-ether to afford 104 in 76% yield; m.p. : 159-161°C (lit.[133b] : 159-161°C); ^{1}H-NMR : δ 7.73 and 7.04 (AA'BB', 4H, C_6H_4), 7.24 (s, 5H, C_6H_5), 5.14 and 4.95 (AB spectrum, 2H, J_{AB} = 12.7 Hz, OCH_2), 3.86 (d, 1H, CH-N), 2.38-1.93 (m, 1H, $CH(CH_3)_2$), 2.28 (s, 3H, $CH_3C_6H_4$), 0.88 (d, 6H, $(CH_3)_2$); exact mass calcd. for D-valine benzyl ester, $C_{12}H_{18}NO_2$ (M^++1), m/e 208.1338, found : 208.1336; chemical-ionization mass spectrum, m/e (relative intensity) : 208 ($[M+1]^+$, H-Val-OBzl, 26%), 173 $(M+1]^+$, TosOH, 98%), 91 ($[C_7H_7]^+$, 91%), 72 (44%); anal. calcd. for $C_{19}H_{25}NO_5S$: C, 60.14; H, 6.64; N, 3.69, found : C, 60.14; H, 6.68; N, 3.63.

3-Bromo-2-benzyloximino-propanoyl-D-valine benzyl ester 105

Triethylamine (5.55 g, 55 mmol) was added dropwise to a stirred and chilled (0°C) solution of 104 (20.85 g, 55 mmol) in 200 ml of CH_2Cl_2. Subsequently, a suspension of 91 (14.96 g, 55 mmol) in 100 ml of CH_2Cl_2 was added to the cold solution. Finally, DCC (Janssen Chimica, 12.0 g, 58 mmol) was added all at once, and stirring was continued at 0°C for 10 minutes and at room temperature overnight. Dicyclohexylurea was then removed by filtration and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in 200 ml of EtOAc, washed with 0.1N HCl and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Column chromatography (solvent system A) of the residue afforded 105 as an oil in 80%

yield. The compound was homogeneous on TLC (solvent system A) : Rf 0.18; ^{1}H-NMR : δ 7.34 (s, 10H, 2x C_6H_5), 7.13 (d, 1H, J = 9.5 Hz, NH), 5.31 (s, 2H, CH_2O), 5.18 and 5.17 (AB spectrum, 2H, CH_2O), 4.64 (double d, 1H, J = 9.5 Hz and 5.0 Hz, CH-N), 4.21 (s, 2H, CH_2Br), 2.44-1.96 (m, 1H, $C\underline{H}(CH_3)_2$), 0.94 and 0.86 (2xd, 6H, $(C\underline{H}_3)_2$); exact mass calcd. for $C_{22}H_{26}BrN_2O_4$ (M^++1), m/e 461.108, found : 461.106; chemical-ionization mass spectrum, m/e (relative intensity) : 553/551 ($[M+C_7H_7]^+$, 16%), 463/461 ($[M+1]^+$, 54%), 381 ($[M-Br]^+$, 26%), 327/325 ($[M-CO_2CH_2C_6H_5]^+$, 15%), 291 ($[M-C_7H_6Br]^+$, 17%), 91 ($[C_7H_7]^+$, 100%).

3-(4-Nitrobenzylthio)-2-benzyloximino-propanoic acid 118

A solution of triethylamine (0.202 g, 2 mmol) in a few ml of dry DME was added dropwise to a stirred solution of 91 (0.273 g, 1 mmol) and 4-nitrobenzyl mercaptan (0.186 g, 1.1 mmol) in dry DME at room temperature and in an argon atmosphere. Stirring was continued until completion of the reaction as monitored by TLC (solvent system F). After filtration the reaction mixture was concentrated in vacuo. The residue was suspended in EtOAc, washed with 0.1N HCl (3 times), H_2O and brine, dried over Na_2SO_4, and concentrated to dryness in vacuo. The residue was dissolved in a minimum amount of EtOAc. Addition of dicyclohexylamine (0.181 g, 1 mmol) resulted in the formation of a white precipitate, the dicyclohexylamine salt of 118. The salt was isolated by filtration, suspended in CH_2Cl_2 and washed with 0.1N HCl (4 times) and H_2O. Subsequently, the organic layer was dried over Na_2SO_4 and concentrated to dryness in vacuo, affording nearly pure 118 in 80% yield. The product could be recrystallized from MeOH/H_2O; m.p. : 141-143°C. The compound was homogeneous on TLC (solvent system F) :

Rf 0.58; ^{1}H-NMR ($CDCl_3/CD_3OD$) : δ 7.94 and 7.29 (AA'BB', 4H, C_6H_4), 7.28 (s, 5H, C_6H_5), 5.26 (s, 2H, OCH_2), 3.75 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.47 (s, 2H, $CH_2C{=}N$).

3-(4-Nitrobenzylthio)-2-benzyloximino-propanoyl-D-valine benzyl ester 108

Entry A : from 118.

Dicyclohexylcarbodiimide (2.27 g, 11 mmol) was added all at once to a solution of 118 (3.60 g, 10 mmol), 104 (4.17 g, 11 mmol) and pyridine (0.87 g, 11 mmol) in EtOAc at 45°C. The mixture was stirred at room temperature for 2 hours, filtered, washed with 0.1N HCl (3 times), H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (solvent system B) affording 108 as an oil in 80% yield.

Entry B : from 105.

A solution of triethylamine (0.464 g, 4.6 mmol) in 5 ml of dry DME was added dropwise to a stirred solution of 105 (1.06 g, 2.3 mmol) and 4-nitrobenzyl mercaptan 93 (0.389 g, 2.3 mmol) in 20 ml of dry DME at room temperature and in an argon atmosphere. Stirring was continued until completion of the reaction (± 3h) as monitored by TLC (solvent system N). The reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2. The solution was washed with 0.1N HCl (2 times) and H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (solvent system B) affording 108 in 91% yield. The compound was homogeneous on TLC (solvent system B) : Rf 0.17; ^{1}H-NMR : δ 8.05 and 7.42 (AA'BB', 4H, C_6H_4), 7.38 (s, 10H, $2xC_6H_5$), 7.18 (d, 1H, NH), 5.24 (s, 2H, OCH_2), 5.21 (AB spectrum, 2H, OCH_2),

4.61 (dd, 1H, J = 9.0 Hz and 4.5 Hz, $NCHCO_2$), 3.77 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.48 (s, 2H, $CH_2C{=}N$), 2.47-2.00 (m, 1H, $C\underline{H}(CH_3)_2$), 0.95 and 0.87 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{32}N_3O_6S$ (M^++1), m/e 550.201, found 550.202; chemical-ionization mass spectrum, m/e (relative intensity) : 550 ($[M+1]^+$, 100%), 442 ($[M-C_7H_7O]^+$, 5%), 414 ($[M-C_8H_7O_2]^+$, 4%), 413 ($[M-C_7H_6NO_2]^+$, 6%), 382 ($[M-C_7H_5NO_2S]^+$, 11%), 170 ($[C_7H_8NO_2S]^+$, 6%), 107 ($[C_7H_7O]^+$, 16%), 106 ($[C_7H_6O]^+$, 17%), 91 ($[C_7H_7]^+$, 90%).

3-Benzylthio-2-benzyloximino-propanoyl-D-valine benzyl ester 109

A solution of triethylamine (1.66 g, 16.4 mmol) in 10 ml of dry DME was added dropwise to a stirred solution of 105 (3.78 g, 8.2 mmol) and freshly distilled benzyl mercaptan 106 (1.02 g, 8.2 mmol) in 80 ml of dry DME at room temperature and in an argon atmosphere. After completion of the reaction (± 3h) as monitored by TLC (solvent system N) the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, washed with 0.1N HCl (2 times) and H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography (solvent system B) to afford pure 109 as an oil in 82% yield. The compound was homogeneous on TLC (solvent system B) : Rf 0.34; ^{1}H-NMR : δ 7.35 (s, 10H, $2xC_6H_5$), 7.23 (s, 5H, C_6H_5), 7.16 (d, 1H, NH), 5.22 (s, 2H, OCH_2), 5.20 and 5.16 (AB spectrum, 2H, J_{AB} = 12.1 Hz, OCH_2), 4.63 (dd, 1H, J = 9.0 Hz and 4.8 Hz, $NCHCO_2$), 3.71 (s, 2H, $C_6H_5C\underline{H}_2S$), 3.51 (s, 2H, $CH_2C{=}N$), 2.46-1.98 (m, 1H, $C\underline{H}(CH_3)_2$), 0.95 and 0.87 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{33}N_2O_4S$ (M^++1), m/e 505.216, found 505.217; chemical-ionization mass spectrum, m/e (relative intensity) : 505 ($[M+1]^+$, 46%), 399 ($[M-C_7H_5O]^+$, 3%), 382 ($[M-C_7H_6S]^+$, 8%), 181 ($[C_{14}H_{13}]^+$, 4%), 137

([C_8H_9S]$^+$, 9%), 123 ([C_7H_7S]$^+$, 6%), 107 ([C_7H_7O]$^+$, 16%), 91 ([C_7H_7]$^+$, 100%).

3-(4-Methoxybenzylthio)-2-benzyloximino-propanoyl-D-valine benzyl ester 110

A solution of triethylamine (7.07 g, 70 mmol) in 70 ml of dry 1,2-dimethoxyethane was added dropwise to a stirred solution of 105 (16.13 g, 35 mmol) and 4-methoxy-α-toluenethiol 107 (Aldrich, 6.47 g, 42 mmol) in dry DME (280 ml) at room temperature and under argon. Stirring was continued until completion of the reaction as monitored by TLC (solvent system B). Triethylamine hydrobromide was removed by filtration and the solvent was evaporated. The residue was dissolved in EtOAc, washed with 0.05N HCl and H_2O, and dried over anhydrous Na_2SO_4. Evaporation of the solvent in vacuo afforded a crude product, which was purified by column chromatography (solvent system B). The product was obtained as an oil in 62% yield. The compound was homogeneous on TLC (solvent system B) : Rf 0.25; ^{1}H-NMR : δ 7.33 (s, 10H, 2xC_6H_5), 7.16 and 6.74 (AA'BB', 4H, C_6H_4), 6.95 (d, 1H, J = 9.0 Hz, NH), 5.20 (s, 2H, CH_2O), 5.20 and 5.15 (AB spectrum, 2H, J_{AB} = 12.0 Hz, CH_2O), 4.62 (double d, 1H, J = 9.0 Hz and 4.8 Hz, CH-N), 3.75 (s, 3H, OCH_3), 3.66 (s, 2H $SCH_2C_6H_4$), 3.48 (s, 2H, $SCH_2C{=}N$), 2.45-1.94 (m, 1H, $CH(CH_3)_2$), 0.94 and 0.86 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{30}H_{35}N_2O_5S$ (M^++1), m/e 535.227, found : 535.228; chemical-ionization mass spectrum m/e (relative intensity) : 656 ([M+$C_8H_{10}O$]$^+$, 1%), 535 ([M+1]$^+$, 7%), 427 ([M-C_7H_7O]$^+$, 2%), 415 ([M-C_8H_7O]$^+$, 1%), 382 ([M-C_8H_8OS]$^+$, 7%), 153 ([C_8H_9OS]$^+$, 11%), 121 ([C_8H_9O]$^+$, 100%), 107 ([C_7H_7O]$^+$, 23%), 91 ([C_7H_7]$^+$, 67%).

N-Benzyloxy-S-(4-nitrobenzyl)-D,L-cysteinyl-D-valine benzyl ester 111

With intervals of 45 minutes seven portions of $(CH_3)_3N.BH_3$, 0.58 g (7.9 mmol) per portion, were added at room temperature to a stirred solution of 108 (2.17 g, 3.95 mmol) in 20 ml of dry dioxane, saturated with dry HCl. After completion of the reaction (± 8h) as monitored by TLC (solvent system F), the solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2, washed with H_2O (3 times) and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography, using successively solvent system B and C, affording 111 as an oily mixture of diastereomers in 30% yield. The mixture showed only one spot on TLC (solvent system C) : Rf 0.38; ^{1}H-NMR : δ 8.12 and 7.41 (AA'BB', 4H, C_6H_4), 7.33 (s, 10H, 2xC_6H_5), 6.10 (t, 1H, NH), 5.15 (s, 2H, CO_2CH_2), 4.73 (s, 2H, $NOCH_2$), 4.73-4.50 (m, 1H, $CHCO_2$), 4.02-3.27 (m, 1H, CHNO), 3.73 and 3.71 (2xs, 2H, $C_6H_4CH_2S$), 3.08-2.47 (m, 2H, $CHCH_2S$), 2.47-1.96 (m, 1H, $CH(CH_3)_2$), 1.08-0.63 (m, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{34}N_3O_6S$ (M^++1), m/e 552.217, found : 552.218; chemical-ionization mass spectrum, m/e (relative intensity) : 552 ($[M+1]^+$, 2%), 446 ($[M-C_7H_5O]^+$, 6%), 399 ($[M-C_7H_6NOS]^+$, 4%), 338 ($[M-C_8H_7NO_4S]^+$, 8%), 170 ($[C_7H_8NO_2S]^+$, 83%), 138 ($[C_7H_8NO_2]^+$, 22%), 136 ($[C_7H_6NO_2]^+$, 21%), 107 ($[C_7H_7O]^+$, 27%), 106 ($[C_7H_6O]^+$, 51%), 91 ($[C_7H_7]^+$, 100%).

N-Benzyloxy-S-benzyl-D,L-cysteinyl-D-valine benzyl ester 112

Compound 112 was prepared from 109 as described above for 111. Purification by column chromatography, using successively solvent system B and C, afforded two fractions of 112, in a total yield of 46%. The first fraction (8%) was homogeneous on TLC (solvent system

C) : Rf 0.54. According to the ^{1}H-NMR spectrum this fraction consisted of one diastereomer (≥ 95%) : δ 7.31 (s, 10H, 2xC_6H_5), 7.23 (s, 5H, C_6H_5), 6.16 (d, 1H, NH), 5.18 and 5.10 (AB spectrum, 2H, J_{AB} = 11.9 Hz, CO_2CH_2), 4.71 (s, 2H, $NOCH_2$), 4.58 (dd, 1H, J = 9.0 Hz and 4.7 Hz, $CHCO_2$), 3.66 (s, 2H, $C_6H_5C\underline{H}_2S$), 3.58-3.37 (5 lines, 1H, CHNO), 2.87 and 2.65 (AB part of ABX spectrum, 2H, J_{AX} = 4.5 Hz, J_{BX} = 9.2 Hz, J_{AB} = 14.3 Hz, $CHC\underline{H}_2S$), 2.45-1.91 (m, 1H, $C\underline{H}(CH_3)_2$), 0.94 and 0.85 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{29}H_{35}N_2O_4S$ (M^++1), m/e 507.232, found : 507.231; chemical-ionization mass spectrum, m/e (relative intensity) : 597 ($[M+C_7H_7]^+$, 1%), 507 ($[M+1]^+$, 47%), 399 ($[M-C_7H_7O]^+$, 10%), 384 ($[M-C_7H_6S]^+$, 12%), 107 ($[C_7H_7O]^+$, 22%), 91 ($[C_7H_7]^+$, 100%).

The other fraction (38%) was also homogeneous on TLC (solvent system C) : Rf 0.54. According to the ^{1}H-NMR spectrum this fraction was enriched in the other diastereomer (up to 85%) of 112. The next NMR-signals were assigned to this compound : δ 7.33 (s, 10H, 2xC_6H_5), 7.25 (s, 5H, C_6H_5), 6.10 (d, 1H, NH), 5.16 (s, 2H, CO_2CH_2), 4.73 (s, 2H, $NOCH_2$), 4.61 (dd, 1H, J = 9.0 Hz and 4.7 Hz, $CHCO_2$), 3.66 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.64-3.42 (5 lines, 1H, CHNO), 2.88 and 2.61 (AB part of ABX spectrum, 2H, J_{AX} = 4.5 Hz, J_{BX} = 9.1 Hz, J_{AB} = 14.3 Hz, $CHC\underline{H}_2S$), 2.40-1.97 (m, 1H, $C\underline{H}(CH_3)_2$), 0.92 and 0.84 (2xd, 6H, $(CH_3)_2$).

N-Benzyloxy-S-(4-methoxybenzyl)-D,L-cysteinyl-D-valine benzyl ester 113

$(CH_3)_3N.BH_3$ (Aldrich) was added at room temperature in seven portions with 45 minutes intervals, 2.19 g (30 mmol) per portion, to a stirred solution of 110 (8.01 g, 15 mmol) in 75 ml of dry dioxane, saturated with dry HCl. After stirring at room temperature for 8-9h, the reaction mixture was concentrated to dryness in

vacuo. The residue was dissolved in CH_2Cl_2, washed with a 5% aqueous $NaHCO_3$ solution and H_2O, dried over anhydrous Na_2SO_4, and concentrated to dryness in vacuo. The residue was purified by column chromatography. Solvent system B was used to elute the borates after which it was changed by solvent system C to elute the desired compound. A mixture of two diastereomers of 113 was obtained as an oil in 22% yield. On TLC (solvent system C) the mixture showed only one spot : Rf 0.46; ^{1}H-NMR : δ 7.33 (s, 11H, 2xC_6H_5 and NH), 7.17 and 6.81 (AA'BB', 4H, C_6H_4), 6.15 (br, 1H, H-NO), 5.15 (s, 2H, CO_2CH_2), 4.73 (s, 2H, N-OCH_2), 4.73-4.50 (m, 1H, $NCHCO_2$), 3.76 (s, 3H, OCH_3), 3.63 (s, 2H, S$CH_2C_6H_4$), 3.63-3.31 (m, 1H, CH-NO), 3.05-2.41 (12 lines, 2x AB part of ABX spectrum, 2H, CH-CH_2S), 2.41-1.95 (m, 1H, $CH(CH_3)_2$), 1.02-0.71 (m, 6H, $(CH_3)_2$); exact mass calcd. for $C_{30}H_{37}N_2O_5S$ (M^++1), m/e 537.242, found: 537.241; chemical-ionization mass spectrum, m/e (relative intensity): 537 ($[M+1]^+$, 5%), 429 ($[M-C_7H_7O]^+$, 3%), 277 ($[M-C_8H_9OS-C_7H_6O]^+$, 8%), 121 ($[C_8H_9O]^+$, 100%), 107 ($[C_7H_7O]^+$, 39%), 91 ($[C_7H_7]^+$, 99%).

3-(4-Methoxybenzylthio)-2-benzyloximino-propanoic acid 119

A solution of 91 (4.08 g, 15 mmol) and 4-methoxy-α-toluenethiol 107 (2.54 g, 16.5 mmol) in 45 ml of dry DME was treated with triethylamine (3.18 g, 31.5 mmol) at room temperature and under argon. After stirring for 16h at room temperature the reaction mixture was filtered and concentrated in vacuo. EtOAc (100 ml) and 1N HCl (40 ml) were added to the residue and the resulting two-phase system was stirred vigorously for a while. Then the layers were separated and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated to dryness in vacuo to afford

crude 119. The product was subjected to column chromatography (solvent system O) to yield pure 119 (75%) as a solid; ^{1}H-NMR: δ 7.34 (s, 5H, C_6H_5), 7.17 and 6.75 (AA'BB', 4H, C_6H_4), 5.28 (s, 2H, OCH_2), 3.76 (s, 3H, OCH_3), 3.69 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.47 (s, 2H, $CH_2C{=}N$); chemical-ionization mass spectrum, m/e (relative intensity): 466 ($[M+C_8H_9O]^+$, 15%), 422 ($[M+C_8H_9O-CO_2]^+$, 2%), 346 ($[M+1]^+$, 1%), 345 (M^+, $C_{18}H_{19}NO_4S$, 2%), 238 ($[M-C_7H_7O]^+$, 3%), 194 ($[M-C_7H_7O-CO_2]^+$, 7%), 153 ($[C_8H_9OS]^+$, 8%), 121 ($[C_8H_9O]^+$, 100%), 107 ($[C_7H_7O]^+$, 4%), 91 ($[C_7H_7]^+$, 18%); positive FAB mass spectrum, m/e (relative intensity) : 346 ($[M+1]^+$, 4%), 121 ($[C_8H_9O]^+$, 32%).

N-Benzyloxy-S-(4-methoxybenzyl)-D,L-cysteine 120

To a solution of 119 (3.61 g, 10.4 mmol) in 50 ml of dry dioxane, saturated with dry HCl, was added $(CH_3)_3N.BH_3$ (1.52 g, 20.8 mmol) at room temperature. After stirring for 1h another portion of $(CH_3)_3N.BH_3$ (1.52 g, 20.8 mmol) was added. After stirring for 16h the reaction was complete as monitored by TLC (solvent system F). The solvent was evaporated in vacuo whereupon the residue was dissolved in EtOAc (100 ml) and washed with 0.1N NaOH (3x70 ml). Subsequently, the combined aqueous layers were acidified immediately with concentrated aqueous hydrochloric acid (12N) to pH 4.4. The resulting precipitate was isolated by filtration and dissolved in CH_2Cl_2; Na_2SO_4 was added to the hazy solution and after stirring for a while, the suspension was filtered through Celite. Concentration of the filtrate in vacuo afforded 120 in 55% yield; ^{1}H-NMR : δ 7.30 (s, 5H, C_6H_5), 7.14 and 6.76 (AA'BB', 4H, C_6H_4), 4.68 (s, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 3.72, 3.63 and 3.56 (3 lines, 3H, $C_6H_4C\underline{H}_2S$ and $C\underline{H}CO_2H$), 2.92-2.48 (AB part of ABX spectrum, 2H, $CHC\underline{H}_2S$); positive FAB mass spectrum, m/e (relative

intensity) : 348 ($[M+1]^+$, $C_{18}H_{22}NO_4S$, 3%), 121 ($[C_8H_9O]^+$, 100%).

N-Acetyl-N-benzyloxy-S-(4-methoxybenzyl)-D,L-cysteinyl-D-valine benzyl ester 123, via N-carboxy anhydride 122

Compound 120 (0.699 g, 2 mmol) and activated charcoal (0.01 g) were suspended in freshly distilled, dry THF (5 ml). At room temperature trichloromethyl chloroformate 121 (Fluka, 0.32 ml, 2.6 mmol) was added all at once to the vigorously stirred suspension. The reaction mixture was stirred at 40°C for two hours. Then it was filtered through Celite and concentrated to dryness in vacuo. The residue, crude 122, was used immediately in the following reaction.

A solution of the crude product in dry CH_2Cl_2 (4 ml) was added dropwise to a stirred and cooled (0°C) solution of 104 (0.76 g, 2 mmol) and triethylamine (0.202 g, 2 mmol) in 10 ml of dry CH_2Cl_2. The reaction mixture was stirred at 0°C for 2h and at room temperature for 16h. Subsequently, a solution of freshly distilled acetyl chloride (0.315 g, 4 mmol) in dry CH_2Cl_2 (1 ml) and a solution of pyridine (0.316 g, 4 mmol) in dry CH_2Cl_2 (2 ml) were added successively at room temperature. After stirring for 5h the solution was washed with H_2O (3 times), dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography (solvent system P) to afford 123 as a mixture of diastereomers in 5% yield, based on 120. The oily mixture was homogeneous on TLC (solvent system C) : Rf 0.32; ^{1}H-NMR : δ 7.34 (s, 10H, 2xC_6H_5), 7.22 and 6.81 (AA'BB', 4H, C_6H_4), 6.98 (d, 1H, NH), 5.18 and 5.13 (AB spectrum and s, 2H, J_{AB} = 12.5 Hz, OCH_2), 5.08-4.64 (m, 3H, OCH_2 and CHNO), 4.55 (dd, 1H, J = 8.5 and 4.5 Hz, $CHCO_2$), 3.76 (s, 3H, OCH_3), 3.66 (s, 2H, $C_6H_4C\underline{H}_2S$), 3.09-2.95 (5 lines, 2H, $CHC\underline{H}_2S$), 2.47-2.00 (m, 1H, $C\underline{H}(CH_3)_2$), 2.18 and 2.15

(2xs, 3H, $CH_3C(O)$), 0.98-0.81 (7 lines, 6H, $(CH_3)_2$); exact mass calcd. for $C_{32}H_{39}N_2O_6S$ (M^++1), m/e 579.253, found : 579.253; chemical-ionization mass spectrum, m/e (relative intensity) : 579 ($[M+1]^+$, 22%), 537 ($[M+1-CH_2CO]^+$, 2%), 471 ($[M-C_7H_7O]^+$, 3%), 443 ($[M-C_8H_7O_2]^+$, 2%), 414 ($[M-C_{10}H_{12}O_2]^+$, 7%), 372 ($[M-C_{12}H_{16}NO_2]^+$, 12%), 211 ($[C_{15}H_{15}O]^+$, 5%), 181 ($[C_{14}H_{13}]^+$, 6%), 121 ($[C_8H_9O]^+$, 100%), 107 ($[C_7H_7O]^+$, 9%), 91 ($[C_7H_7]^+$, 31%).

N-Acetyl-N-benzyloxy-S-benzyl-D,L-cysteinyl-D-valine benzyl ester 124

A cooled (0°C) solution of 112 (mixture of diastereomers (1 : 1), 0.205 g, 0.4 mmol) and acetyl chloride (0.035 g, 0.4 mmol) in dry CH_2Cl_2 (5 ml) was treated with a solution of pyridine (0.035 g, 0.4 mmol) in dry CH_2Cl_2 (2 ml). After stirring at room temperature for 4h the reaction was complete as monitored by TLC (solvent system F). The reaction mixture was washed with 0.05N HCl, H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (solvent system P) to afford 124 as a mixture of diastereomers (1 : 1) in 42% yield. This mixture, an oil, was homogeneous on TLC (solvent system P): Rf 0.20; ^{1}H-NMR: δ 7.31 (s, 5H, C_6H_5), 7.28 (s, 5H, C_6H_5), 7.24 (s, 5H, C_6H_5), 6.95 (d, 1H, NH), 5.15 and 5.10 (AB spectrum and s, 2H, J_{AB}=12.9 Hz, OCH_2), 5.06-4.61 (m, 3H, OCH_2 and CHNO), 4.53 (dd, 1H, J=8.6 Hz and 4.6 Hz, $CHCO_2$), 3.68 (s, 2H, $C_6H_5C\underline{H}_2S$), 3.09-2.95 (5 lines, 2H, $CHC\underline{H}_2S$), 2.44-1.97 (m, 1H, $C\underline{H}(CH_3)_2$), 2.16 and 2.13 (2xs, 3H, $CH_3C(O)$), 0.98-0.80 (7 lines, 6H, $(CH_3)_2$); exact mass calcd. for $C_{31}H_{37}N_2O_5S$ (M^++1), m/e 549.242, found: 549.241; chemical-ionization mass spectrum, m/e (relative intensity): 549 ($[M+1]^+$, 20%), 507 ($[M+1-CH_2CO]^+$, 6%), 443 ($[M-C_7H_5O]^+$, 4%), 425 ($[M-C_7H_7S]^+$, 6%), 384 ($[M-C_9H_{10}NO_2]^+$, 10%), 342 ($[M-C_{12}H_{16}NO_2]^+$,

22%), 319 ($[M-C_{14}H_{13}OS]^+$, 42%), 229 ($[C_{14}H_{13}OS]^+$, 4%), 208 ($[C_{12}H_{18}NO_2]^+$, 14%), 139 ($[C_7H_7OS]^+$, 9%), 107 ($[C_7H_7O]^+$, 58%), 91 ($[C_7H_7]^+$, 100%).

N-Benzyloxycarbonyl-L-glutamic acid 127

Benzyl chloroformate (1.74 g, 10.2 mmol) was added dropwise to a vigorously stirred solution of L-glutamic acid 125 (1.0 g, 6.8 mmol) in 30 ml of aqueous 1M $NaHCO_3$ at room temperature. After stirring for 4h the reaction mixture was washed twice with ether and subsequently cooled to 0°C. Then the aqueous alkaline solution was acidified to pH 3-4 by adding cautiously concentrated aqueous hydrochloric acid to the vigorously stirred solution. The product separated as an oil. The aqueous layer was decanted and washed thrice with EtOAc. The oil was dissolved in EtOAc and washed with H_2O and brine. The combined EtOAc layers were dried over Na_2SO_4 and concentrated in vacuo to leave an oil (76%) which solidified upon prolonged storage in a refrigerator. The product was, although not recrystallized, homogeneous on TLC (solvent system D): Rf 0.38; m.p.: 118-120°C (lit.[154]: 120-121°C); ^{1}H-NMR: δ 7.32 (s, 5H, C_6H_5), 5.59 (br.d., 1H, NH), 5.10 (s, 2H, OCH_2), 4.62-4.24 (m, 1H, CH $(CH_2)_2$), 2.67-1.95 (m, 4H, $(CH_2)_2$); exact mass calcd. for $C_{13}H_{16}NO_6$ (M^++1), m/e 282.0978, found: 282.0974; chemical-ionization mass spectrum, m/e (relative intensity): 328 ($[M+47]^+$, 3%) 282 ($[M+1]^+$, 3%), 238 ($[M+1-CO_2]^+$, 21%), 220 ($[M-CO_2-OH]^+$, 8%), 130 ($[C_5H_8NO_3]^+$, 87%), 107 ($[C_7H_7O]^+$, 9%), 91 ($[C_7H_7]^+$, 100%); anal. calcd. for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98, found: C, 54.87; H, 5.26; N, 4.96.

α-Benzyl N-benzyloxycarbonyl-L-glutamate 129 [101b,c]

Dicyclohexylamine (3.22 g, 17.8 mmol) was added to a solution of 127 (5.0 g, 17.8 mmol) in 15 ml of dry DMF. The precipitated dicyclohexylamine salt of 127 was collected by filtration and dried. Subsequently the compound was dissolved in 15 ml of dry DMF at 45°C. Benzyl bromide (3.04 g, 17.8 mmol) was added dropwise to the solution, resulting in the precipitation of dicyclohexylamine hydrobromide. After stirring for a while the reaction mixture was filtered and concentrated in vacuo. The residue was dissolved in EtOAc (25 ml) and stored in a refrigerator for 24h. Filtration followed by evaporation of the solvent in vacuo afforded 129 as an oil in 80% yield; ^{1}H-NMR: δ 7.26 (s, 10H, 2xC_6H_5), 5.65-5.32 (br., 1H, NH), 5.11 and 5.06 (2xs, 4H, 2xOCH_2), 4.60-4.17 (m, 1H, C<u>H</u>$(CH_2)_2$), 2.58-1.77 (m, 4H, $(CH_2)_2$); exact mass calcd. for $C_{20}H_{22}NO_6$ (M^++1), m/e 372.1447, found: 372.1440; chemical-ionization mass spectrum, m/e (relative intensity): 418 ($[M+47]^+$, 4%), 372 ($[M+1]^+$, 7%), 328 ($[M+1-CO_2]^+$, 26%), 310 ($[M-CO_2-OH]^+$, 7%), 220 ($[M-C_8H_7O_3]^+$, 12%), 181 ($[C_{14}H_{13}]^+$, 19%), 129 ($[C_5H_7NO_3]^+$, 21%), 107 ($[C_7H_7O]^+$, 7%), 91 ($[C_7H_7]^+$, 100%); IR ($CHCl_3$): 3500-2500, 3435, 1740, 1715, 1510 and 1215cm^{-1}.

α-Benzyl N-benzyloxycarbonyl-L-glutamate dicyclohexylamine salt 130 [101b,c]

Dicyclohexylamine (2.29 g, 12.6 mmol) was added dropwise to a cooled (0°C) solution of 129 (4.27 g, 11.5 mmol) in 20 ml of dry EtOAc. After stirring for 1h the precipitate was collected by filtration, washed with cold EtOAc, and dried thoroughly in a vacuum desiccator over KOH, affording 130 in quantitative yield. The product was recrystallized from EtOH; m.p.: 161-163°C

(lit.[101b,c]: 162-164°C); $[\alpha]_D^{25}$= -12.2°, c=2.1; MeOH (lit.[139] $[\alpha]_D^{25}$= -12.0°, c=2.1; MeOH); ^{1}H-NMR: δ 7.31 (s, 10H, 2x C_6H_5), 7.09 (d, 2H, H_2N^+), 6.08 (br.d., 1H,NH), 5.16 and 5.05 (2xs, 4H, 2x OCH_2), 4.46-3.91 (m, 1H, $C\underline{H}(CH_2)_2$), 2.88 (m, 2H, CH-N-CH), 2.49-0.77 (m, 24H, $(CH_2)_2$ and $N(CHC_5\underline{H}_{10})_2$).

Formation of benzyl N-benzyloxycarbonyl-L-pyroglutamate 132 in an attempt to prepare N-benzyloxycarbonyl-L-γ-glutamyl chloride α-benzyl ester 131

Freshly distilled oxalyl chloride (1.03 g, 8.1 mmol) was added dropwise within 10 minutes to a stirred and cooled (0°C) solution of 129 (2.02 g, 5.4 mmol) and DMF (two drops) in 6 ml of dry CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 h. Evaporation of the solvent in vacuo afforded a solid, which turned out to be *benzyl N-benzyloxycarbonyl-L-pyroglutamate 132*. The product was not recrystallized; m.p.: 105-109°C (lit.[141,142] : 108-109°C); ^{1}H-NMR: δ 7.33 (s, 10H, 2xC_6H_5), 5.22 (s, 2H, OCH_2), 5.13 (s, 2H, OCH_2), 4.81-4.61 (m, 1H, $C\underline{H}(CH_2)_2$), 2.96-1.80 (m, 4H, $(CH_2)_2$); IR ($CHCl_3$): 1795, 1750 and 1725 cm^{-1}; exact mass calcd. for $C_{20}H_{20}NO_5$ (M^++1), m/e 354.1341, found: 354.1340; chemical-ionization mass spectrum, m/e (relative intensity): 354 ($[M+1]^+$, 5%), 338 ($[M+1-O]^+$, 4%), 310 ($[M+1-CO_2]^+$, 26%), 220 ($[M-C_8H_5O_2]^+$, 14%), 181 ($[C_{14}H_{13}]^+$, 27%), 107 ($[C_7H_7O]^+$, 19%), 91 ($[C_7H_7]^+$, 100%); anal. calcd. for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96, found: C, 67.39; H, 5.27; N, 4.04.

N-(Benzyloxycarbonyl)-L-α-aminoadipic acid 128

Benzyl chloroformate (Aldrich, 7.16 g, 42 mmol) was added dropwise to a stirred solution of L-α-aminoadipic acid 126 (Sigma,

4.83 g, 30 mmol) in 102 ml of 1N NaOH. The temperature of the reaction mixture was kept below 10°C by means of an ice-bath. During the course of the reaction the pH of the solution was monitored and kept at pH 11 by addition of an aqueous 1N NaOH solution.

After completion of the reaction, as monitored by the ninhydrin test*, the reaction mixture was washed twice with ether. In order to precipitate the product the aqueous alkaline solution was acidified to pH 2-3 by adding cautiously concentrated hydrochloric acid to the vigorously stirred and cooled (0°C) solution. The precipitated product 128 was filtered and dried. The filtrate was extracted with several portions of EtOAc. The combined extracts were washed with H_2O and brine, dried over Na_2SO_4, and concentrated to dryness in vacuo to leave a second crop of the product. Compound 128, which was homogeneous on TLC (Rf: 0.39, solvent system D), was obtained in a yield of 90%. Recrystallization from MeOH/H_2O gave small needles, m.p.: 134-136°C; ^{1}H-NMR (CD_3OD): δ 7.38 (s, 5H, C_6H_5), 5.15 (s, 2H, CH_2O), 4.33-4.05 (m, 1H, CH-N), 2.36 (m, 2H, CH_2COOH), 2.05-1.53 (m, 4H, $CH_2CH_2CH_2CO_2H$); exact mass calcd. for $C_{14}H_{18}NO_6$ (M^++1), m/e 296.1134, found: 296.1130; chemical-ionization mass spectrum, m/e (relative intensity): 342 ($[M+47]^+$, 22%), 296 ($[M+1]^+$, 8%), 252 ($[M+1-CO_2]^+$, 30%), 144 ($[C_6H_{10}NO_3]^+$, 28%), 91 ($[C_7H_7]^+$, 100%); anal. calcd. for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74, found: C, 56.55; H, 5.77; N, 4.73.

* Take a sample (a few drops) of the reaction mixture, acidify it to pH 5 with acetic acid and add it to 1 ml of a ninhydrin solution, which is prepared by dissolving ninhydrin (0.12 g) in a mixture of 200 ml of n-BuOH and 8 ml of glacial acetic acid. If heating of the

resulting solution leads to a blue-violet colouration, which indicates the presence of an amino acid, the reaction is not complete. Sometimes an additonal amount of benzyl chloroformate has to be added.

L-4-[3-(Benzyloxycarbonyl)-5-oxo-4-oxazolidinyl]-butanoic acid 135

A suspension of N-(benzyloxycarbonyl)-L-α-aminoadipic acid 128 (2.95 g, 10 mmol), p-toluenesulfonic acid monohydrate (0.114 g, 0.6 mmol) and paraformaldehyde (0.81 g, 27 mmol) in 100 ml of toluene was heated under reflux. A Dean-Stark separator was used to trap the azeotropically removed water. After 45 minutes the reaction mixture was cooled, filtered and diluted with 10 ml of EtOAc. Neutralization of the diluted filtrate was accomplished by adding a solution of Na_2CO_3 (0.032 g, 0.3 mmol) in 1 ml of H_2O. After stirring for 30 minutes the resulting mixture was extracted three times with H_2O (1 ml). Then the solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2. The solution was dried over anhydrous Na_2SO_4 and concentrated to dryness in vacuo to leave an oil, which was not entirely homogeneous on TLC (solvent system D): Rf 0.62. The crude product (yield 98%) was used in the next reaction without further purification; $[\alpha]_D^{20}$ = +82.0° (c= 1.0, $CHCl_3$); ^{1}H-NMR: δ 7.33 (s, 5H, C_6H_5), 5.52 (d, 1H, O-CH-N), 5.24 and 5.17 (2xs, 3H, $C_6H_5C\underline{H}_2$ and O-CH-N), 4.42-4.17 (m, 1H, NC$\underline{H}$CH$_2$), 2.52-2.19 (m, 2H, $C\underline{H}_2CO_2H$), 2.19-1.37 (m, 4H, $C\underline{H}_2C\underline{H}_2CH_2CO_2H$); exact mass calcd. for $C_{15}H_{18}NO_6$ (M^++1), m/e 308.1134, found: 308.1129; chemical-ionization mass spectrum, m/e (relative intensity): 354 ($[M+1+CH_2O_2]^+$, 100%), 308 ($[M+1]^+$, 18%), 264 ($[M+1-CO_2]^+$, 50%), 91 ($[C_7H_7]^+$, 75%); IR ($CHCl_3$): 1805 (A) and 1715 cm^{-1} (B), A<B.

L-4-[3-(Benzyloxycarbonyl)-5-oxo-4-oxazolidinyl]-butanoyl chloride 136

PCl_5 (2.08 g, 10 mmol) was added all at once to a stirred and cooled (0°C) solution of 135 (3.07 g, 10 mmol) in 35 ml of dry toluene. Stirring was continued at 0°C for a few minutes and then at room temperature for 25 minutes. The clear solution was filtered to remove a jelly-like material (only a small quantity) and concentrated to dryness in vacuo. During this procedure the temperature of the heating bath was kept below 20°C. To remove residual $POCl_3$ dry toluene was added to the residue and the mixture was evaporated again; this was repeated twice. The crude acid chloride (yield: 3.12 g) was used in the next reaction without further purification. Its purity was about 87% as estimated from the NMR spectrum; ^{1}H-NMR: δ 7.35 (s, 5H, C_6H_5), 5.52 (d, 1H, O-CH-N), 5.25 and 5.18 (2xs, 3H, $C_6H_5CH_2$ and O-CH-N), 4.42-4.16 (m, 1H, $NCHCH_2$), 3.11-2.72 (m, 2H, $CH_2C(O)Cl$), 2.19-1.46 (m, 4H, $CH_2CH_2CH_2C(O)Cl$); chemical-ionization mass spectrum, m/e (relative intensity): 374/372 ($[M+1+CH_2O_2]^+$, 69%), 326 ($[M+1]^+$, $C_{15}H_{17}ClNO_5$, 2%), 290 ($[M-Cl]^+$, 3%), 282/284 ($[M+1-CO_2]^+$, 61%), 246 ($[M-Cl-CO_2]^+$, 34%), 156 ($[C_7H_{10}NO_3]^+$, 58%), 91 ($[C_7H_7]^+$, 100%); IR ($CHCl_3$): 1800 (A) and 1720 cm^{-1} (B), A≥B.

N-[L-4-(3-Benzyloxycarbonyl-5-oxo-4-oxazolidinyl)-butanoyl],N-benzyloxy-S-benzyl-D,L-cysteinyl-D-valine benzyl ester 138

A solution of 112 (1.52 g, 3 mmol) in 9 ml of dry CH_2Cl_2 was added to a cooled (0°C) and stirred solution of crude 136 (2.17 g, ± 5.8 mmol) in 11 ml of dry CH_2Cl_2. Then the stirred and cooled mixture was supplied with a catalytic amount of 4-dimethylamino-pyridine. Subsequently, a solution of dry pyridine (0.46 g, 5.8 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise at 0°C. The reaction

mixture was stirred at room temperature until completion of the reaction (45 minutes) as monitored by TLC (solvent system E) and then washed with 0.05N HCl (2x 20 ml) and H_2O (20 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo.

When the less polar diastereomer of 112 had been used as starting material, purification of the residue by column chromatography (solvent system E) afforded a pure diastereomer of 138 in 81% yield. This compound, an oil, was homogeneous on TLC (solvent system E): Rf 0.18; ^{1}H-NMR: δ 7.34, 7.32, 7.27 and 7.23 (4xs, 20H, 4xC_6H_5), 6.93 (d, 1H, J=8.6 Hz, NH), 5.51 (d, 1H, J=4.5 Hz, OCHN), 5.22, 5.16 and 5.11 (3xs, 5H, OCHN and 2xOCH_2), 5.05-4.61 (m, 3H, C<u>H</u>NOC<u>H</u>$_2$), 4.53 (dd, 1H, J=8.6 Hz and 4.5 Hz, C<u>H</u>NH), 4.41-4.17 (m, 1H, C<u>H</u>$(CH_2)_3$), 3.67 (s, 2H, C_6H_5C<u>H</u>$_2$S), 3.01 (d, 2H, J=7.7 Hz, CHC<u>H</u>$_2$S), 2.86-1.38 (m, 7H $(CH_2)_3$ and C<u>H</u>$(CH_3)_2$), 0.89 (t, 6H, $(CH_3)_2$); positive FAB mass spectrum, m/e (relative intensity): 796 ($[M+1]^+$, $C_{44}H_{50}N_3O_9S$, 5%), 582 ($[M-C_{14}H_{13}S]^+$, 2%), 507 ($[C_{29}H_{35}N_2O_4S]^+$, 3%), 384 ($[M-C_{22}H_{21}NO_5S]^+$, 3%), 181 ($[C_{14}H_{13}]^+$, 10%), 107 ($[C_7H_7O]^+$, 5%), 91 ($[C_7H_7]^+$, 100%).

When a mixture of diastereomers of 112, enriched (80-90%) in the more polar diastereomer, had been used as starting material, the residue was purfied by column chromatography, applying successively solvent system B and E. This procedure afforded the other (more polar) diastereomer of 138 in 66% yield. The compound, also an oil, was homogeneous on TLC (solvent system E): Rf 0.18; ^{1}H-NMR: δ 7.33 and 7.26 (2xs, 20H, 4xC_6H_5), 6.91 (d, 1H, J=8.6 Hz, NH), 5.51 (d, 1H, J=4.6 Hz, O-CH-N), 5.23 and 5.16 (2xs, 5H, O-CH-N and 2xOCH_2), 5.06-4.62 (m, 3H, C<u>H</u>NOC<u>H</u>$_2$), 4.53 (dd, 1H, J=8.6 Hz and 4.6 Hz, C<u>H</u>NH), 4.41-4.18 (m, 1H, C<u>H</u>$(CH_2)_3$), 3.68 (s, 2H,

$C_6H_5C\underline{H}_2S$), 3.04 (d, 2H, J=7.6 Hz, $CHC\underline{H}_2S$), 2.60-1.42 (m, 7H, $(CH_2)_3$ and $C\underline{H}(CH_3)_2$), 0.90 and 0.82 (2xd, 6H, $(CH_3)_2$); positive FAB mass spectrum, m/e (relative intensity): 796 ($[M+1]^+$, $C_{44}H_{50}N_3O_9S$, 3%), 582 ($[M-C_{14}H_{13}S]^+$, 2%), 507 ($[C_{29}H_{35}N_2O_4S]^+$, 3%), 384 ($[M-C_{22}H_{21}NO_5S]^+$, 2%), 181 ($[C_{14}H_{13}]^+$, 6%), 107 ($[C_7H_7O]^+$, 4%), 91 ($[C_7H_7]^+$, 100%).

N-[L-4-(3-Benzyloxycarbonyl-5-oxo-4-oxazolidinyl)-butanoyl],N-benzyloxy-S-(4-methoxybenzyl)-D,L-cysteinyl-D-valine benzyl ester 139

A solution of 113 (1.60 g, 3 mmol) in 9 ml of dry CH_2Cl_2 was added to a cooled (0°C) and stirred solution of crude 136 (1.95 g, ± 5.2 mmol) in 10 ml of dry CH_2Cl_2. Then the stirred and cooled mixture was supplied with a catalytic amount of 4-dimethylamino-pyridine. Subsequently, a solution of dry pyridine (0.41 g, 5.2 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise at 0°C. The reaction mixture was stirred at room temperature until completion of the reaction (45 minutes) as monitored by TLC (solvent system E) and then washed with 0.05N HCl (2x20 ml) and H_2O (20 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness in vacuo. The residue was purified by column chromatography, using successively solvent system E and C as eluant. This procedure afforded one pure diastereomer of 139 (the less polar one) in 9% yield and a mixture of two diastereomers of 139 in 49% yield. The pure diastereomer (an oil) was homogeneous on TLC (solvent system E): Rf 0.12; ^{1}H-NMR: δ 7.32, 7.31 and 7.27 (3xs, 15H, $3xC_6H_5$), 7.16 and 6.78 (AA'BB', 4H, C_6H_4), 6.91 (d, 1H, NH), 5.53 (d, 1H, J=4.5 Hz, O-CH-N), 5.23, 5.17 and 5.10 (3xs, 5H, O-CH-N and 2x $C(O)-OCH_2$), 5.10-4.62 (m, 3H, $C\underline{H}_2ONC\underline{H}$), 4.53 (dd, 1H, J=8.5 Hz and 4.5 Hz, $C\underline{H}NH$), 4.42-4.21 (m, 1H, $C\underline{H}(CH_2)_3$), 3.76 (s, 3H, OCH_3),

3.64 (s, 2H, $SC\underline{H}_2C_6H_4$), 3.00 (d, 2H, J=7.7 Hz, $CHC\underline{H}_2S$), 2.69-1.37 (m, 7H, $(CH_2)_3$ and $C\underline{H}(CH_3)_2$), 0.90 (t, 6H, $(CH_3)_2$); positive FAB mass spectrum, m/e (relative intensity): 826 ($[M+1]^+$, $C_{45}H_{52}N_3O_{10}S$, 6%), 537 ($[C_{30}H_{37}N_2O_5S]^+$, 2%), 381 (2%), 121 ($[C_8H_9O]^+$, 100%), 91 ($[C_7H_7]^+$, 100%); negative FAB: 824 ($[M-1]^-$, $C_{45}H_{50}N_3O_{10}S$, 2%), 734 ($[M-C_7H_7]^-$, 20%), 690 ($[M-C_7H_7-CO_2]^-$, 19%), 153 ($[C_8H_9OS]^-$, 48%), 91 ($[C_7H_7]^-$, 100%).

The mixture of diastereomers (an oil) gave only <u>one</u> spot on TLC (solvent system E): Rf 0.12; ^{1}H-NMR: δ 7.32 (s, 15H, $3xC_6H_5$), 7.18 and 6.80 (AA'BB', 4H, C_6H_4), 7.00 (d, 1H, NH), 5.52 (d, 1H, O-CH-N), 5.22-5.04 (m, 5H, O-CH-N and $2xC(O)OCH_2$), 5.04-4.64 (m, 3H, CH_2ON and $C\underline{H}CH_2S$), 4.64-4.42 (m, 1H, $C\underline{H}NH$), 4.42-4.16 (m, 1H, $C\underline{H}(CH_2)_3$), 3.76 (s, 3H, OCH_3), 3.64 (s, 2H, $SC\underline{H}_2C_6H_4$), 3.11-2.87 (2xd, 2H, $CHC\underline{H}_2S$), 2.61-1.38 (m, 7H, $(CH_2)_3$ and $C\underline{H}(CH_3)_2$), 1.04-0.70 (m, 6H, $(CH_3)_2$).

N-[δ-(L-α-Aminoadipyl)], N-hydroxy-L-cysteinyl-D-valine, disulfide <u>144</u>

A 1M solution of $B(CF_3COO)_3$ in CF_3COOH (8 ml) was added to a cooled (0°C) solution of the pure diastereomer <u>139</u> (0.200 g, 0.24 mmol) in 4 ml of CF_3COOH. After stirring for 3h at room temperature, the excess of CF_3COOH was evaporated in vacuo. H_2O (40 ml) and CH_2Cl_2 (40 ml) were added to the residue and the resulting two-phase system was vigorously stirred at room temperature until two clear layers were obtained. After separation of the layers the aqueous solution was freeze-dried. The residue was purified by ion exchange chromatography (18 x 1 cm column, Dowex 50X8-400, H^+, Janssen Chimica) with a H_2O-1M aq. pyridine gradient as eluant. The $FeCl_3$-positive fractions were combined and lyophilized to afford 37.6 mg of the crude product. The crude product (22.6 mg) was

dissolved in 5 ml of H_2O. The pH of the solution was adjusted to 8-9 by adding a few drops of a diluted aqueous ammonia solution. Oxygen was bubbled through the solution for 4 hours after which the solution was freeze-dried. The residue was purified by reversed phase chromatography (octadecylsilane), using MeOH/25 mM NH_4HCO_3 (20:80, v/v) as eluant. This afforded pure 144 in 2% yield; ^{1}H-NMR (500 MHz, D_2O): δ 5.22 (4 lines, X part of ABX spectrum, 1H, CHNO), 3.92 (d, 1H, CHNH), 3.53 (t, 1H, H_2NCHCH_2), 3.17-2.89 (8 lines, AB part of ABX spectrum, 2H, CH_2S), 2.56-2.34 (m, 2H, $CH_2C(O)N$), 2.01-1.84 (m, 1H, $CH(CH_3)_2$), 1.79-1.60 (m, 2H, $CHCH_2CH_2$), 1.60-1.39 (m, 2H, $CH_2CH_2CH_2$), 0.70 and 0.67 (2xd, 6H, $(CH_3)_2$); positive FAB mass spectrum, m/e: 779 ($[M+Na]^+$, $C_{28}H_{48}N_6NaO_{14}S_2$), 757 ($[M+1]^+$), 741 ($[M+1-O]^+$), 380 ($[C_{14}H_{26}N_3O_7S]^+$, monomer), 144 ($[C_6H_{10}NO_3]^+$), 98 ($[C_5H_8NO]^+$), 72 ($[C_4H_{10}N]^+$).

Incubation and bioassay

The incubation of 144 (600 µg) with a partially purified preparation of isopenicillin N synthetase was carried out in the Dyson Perrins Laboratory (Prof. J.E. Baldwin, Oxford) according to the procedure described by Baldwin and co-workers.[36,46c] $Fe(II)SO_4$ (5 mM), ascorbic acid (50 mM), DTT (100 mM) and catalase (1/10 suspension) were added as cofactors. The pH of the incubation mixture was adjusted to 7.5-8.0, using 150 mM aq. NaOH.

Bioassays were carried out by the hole-plate method.[17,148]

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CHAPTER V

REDUCTION OF OXIMES TO N-HYDROXY-α-AMINO ACID DERIVATIVES USING $NaBH_3CN$

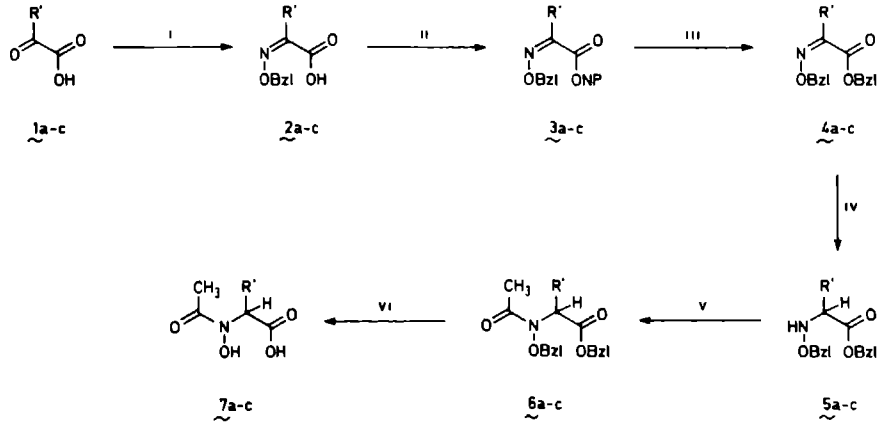
REDUCTION OF OXIMES TO N-HYDROXY-α-AMINO ACID DERIVATIVES USING $NaBH_3CN$

5.1 *INTRODUCTION*

The chemistry of naturally occurring α-amino acids is well established and their biochemical importance is fully understood. Much less is known, however, about the chemical and biochemical behaviour of non-protein amino acids like N-hydroxy-α-amino acids, α,β-dehydroamino acids and α- or β-functionalized amino acids. The chemistry of N-hydroxy-α-amino acids and derivatives thereof has been the subject of a review.[1] A versatile approach for the synthesis of this class of compounds, featuring the reduction of α-oximino acid derivatives, has been developed in our laboratories.[2] This method has been employed by us for the synthesis of some racemic N-acetyl-N-hydroxy-α-amino acids 7 as shown in scheme I.[3]

The hydroxamic acids 7a, 7b and 7c were obtained in 34%, 37% and 32% overall yield respectively from the relevant α-ketocarboxylic acids 1. The crucial step involves reduction of the α-oximino acid derivatives 4a-c to the corresponding N-hydroxy-α-amino acid derivatives 5a-c using an amine-borane complex in the presence of a large excess of hydrochloric acid. This method[2] works well when applied to simple compounds like 4a-c. Although more complex N-hydroxy amino acid derivatives, like for example the N-hydroxy cysteine-derived peptides discussed in chapter IV, have also been synthesized in this way, the results were in general less satisfying. A large excess of the reducing agent is needed to

Scheme I



a R'=CH_3 b R'=CH_2CH_3 c R'=$CH(CH_3)_2$

i $C_6H_5CH_2ONH_2.HCl$, H_2O

ii 4-$O_2NC_6H_4OH$, DCC, EtOAc

iii $C_6H_5CH_2OH$, DBU, CH_2Cl_2

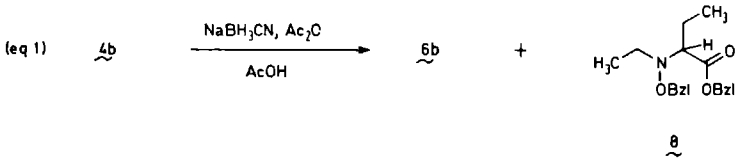
iv $(CH_3)_3N.BH_3$, HCl, Et_2O

v $CH_3C(O)Cl$, pyridine, CH_2Cl_2

vi Pd(C), H_2, CH_3OH

achieve complete conversion of the starting material and numerous by-products are formed. This makes purification of the crude product a laborious job, affording the desired compound in moderate to low yield.

In an attempt to shorten the synthesis depicted in scheme I, a one-step conversion of 4b into 6b by treatment of 4b with $NaBH_3CN$ in acetic acid containing an excess of acetic anhydride was studied (eq.1).[4] Besides a considerable amount of the starting material 4b (44%) and the desired product 6b (36%) compound 8 was isolated in 20% yield. Probably, the aldehyde needed for the reductive alkylation

(eq 1) 

underlying the formation of the latter compound, is generated by partial reduction of the solvent.[5]

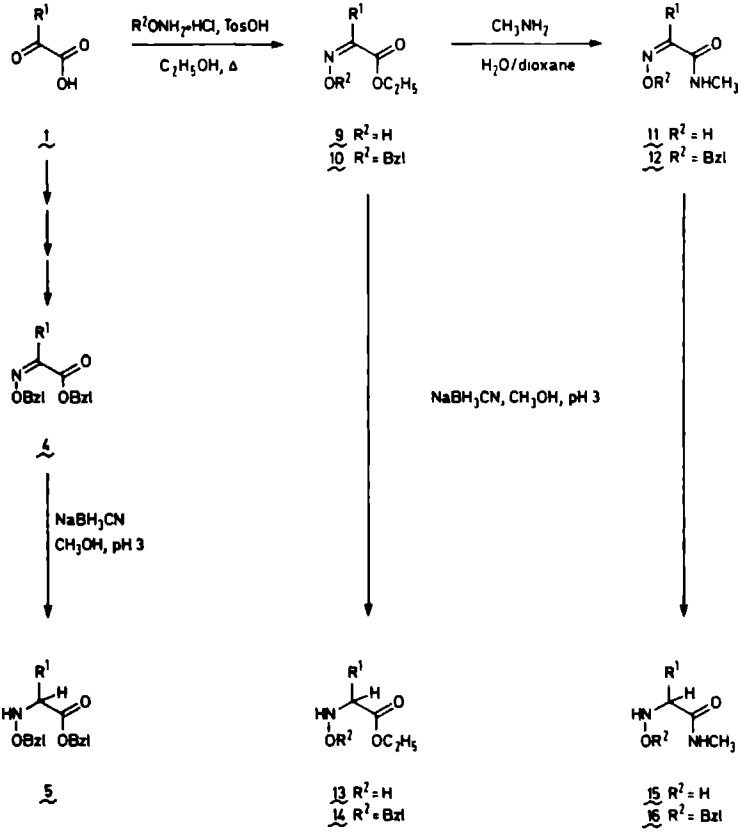
Although the conversion was far from complete, the experiment showed that, as contrasted with earlier observations,[6] $NaBH_3CN$ is capable of reducing the carbon-nitrogen double bond of α-oximino esters. We anticipated that due to milder reaction conditions and easier removal of the excess of the reducing agent, $NaBH_3CN$ might be an attractive alternative for the amine-borane complexes, especially in the synthesis of N-hydroxy peptides (see chapter IV). In this chapter the results of our attempts to develop procedures for the synthesis of the title compounds from the corresponding α-oximino acid derivatives by means of $NaBH_3CN$, are presented.

5.2 *REDUCTION OF α-OXIMINO ACID DERIVATIVES WITH $NaBH_3CN$*

The starting materials (4,9-12) were prepared according to known procedures (scheme I/II).[2a-c]

It has been demonstrated that use of a carboxylic acid (formic or acetic acid) as solvent for the reduction of α-oximino esters with $NaBH_3CN$ leads to a mixture of products, since reductive alkylation of the product takes place (see eq.1 and section 5.3) in addition to reduction of the oxime double bond. So, to achieve selective formation of primary N-hydroxy amino acid derivatives another solvent had to be chosen. Methanol seemed to be most

Scheme II



1-7, 9-16	a	b	c	d	e	f
R^1	CH_3	CH_2CH_3	i-C_3H_7	H	C_6H_5	$C_6H_5CH_2$

convenient for this purpose.[7] We observed that it is necessary to carry out the reaction at about pH 3. Obviously, hydride transfer is preceded by protonation of the oxime. Because the reduction consumes acid, methyl orange was used as indicator to monitor the change in pH during the reaction.[8] The pH was held at the red-orange transition point ("pH 3") by the dropwise addition of 7N methanolic HCl.

The results of the reductions are summarized in table 1; in the second and third column the results of comparable reductions with pyridine- and trimethylamine-borane complexes, carried out by J.D.M. Herscheid *et.al.*[2a-c], are mentioned.

Table 1

	R^1	$NaBH_3CN$[a]	pyridine-borane[a]	trimethylamine-borane[a]
5c	i-C_3H_7	67[b] (15)	--	65[c] (2.5)
13a	CH_3	90[b] (6)	100[b], 75[c] (3)	58[c] (1)
14a	CH_3	88[b,d] (15)	94[c] (3)	100[b] (1)
14c	i-C_3H_7	95[b] (15)	--	--
14d	H	84[b] (13)	95[c] (3)	100[b] (1)
14e	C_6H_5	15[b] (15)	0[b] (3)	65[c] (4)
14f	$C_6H_5CH_2$	56[b] (15)	50[c] (3)	80[c] (2)
15a	CH_3	40[b] (15)	85[b] (5)	40[c] (1)
16a	CH_3	30[b] (15)	65[b] (3)	100[b], 89[c] (2)
16f	$C_6H_5CH_2$	0[b] (15)	0[b] (3)	94[c] (8)

a: The number of equivalents of the reducing agent used is given in parentheses.

b: Yield (%) estimated from the ^{1}H-NMR spectrum.

c: Yield (%) of product isolated by preparative HPLC.

d: Neither the reaction rate nor the yield (83%[b]) was substantially influenced by the addition of water to the reaction mixture (12.5%, v).

Because the reactions proceeded quite cleanly, they could be monitored by ^{1}H-NMR spectroscopy. The results indicate that in

general $NaBH_3CN$ is inferior to the borane complexes. The advantages associated with the use of $NaBH_3CN$, i.e. mild reaction conditions and easy removal of the excess of the reducing agent by a simple extraction procedure, are counteracted by incomplete conversions, which then requires separation of the product from the starting material in an additional purification step. Attempts to reduce peptides having an O-protected α-oximino function, e.g. compounds 94 and 109 described in chapter IV, with $NaBH_3CN$ to the corresponding N-hydroxy peptide derivatives failed completely.

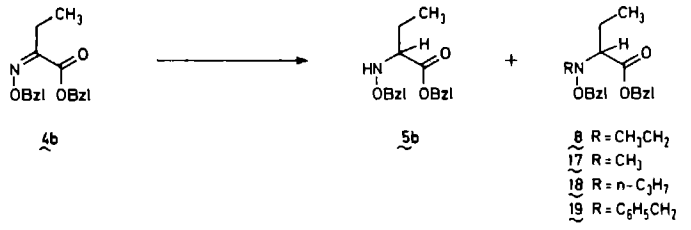
5.3 *SYNTHESIS OF SECONDARY N-BENZYLOXY-α-AMINO ACID DERIVATIVES BY REDUCTIVE ALKYLATION*

As indicated in eq.1 it is possible to obtain secondary N-benzyloxy-α-amino acid esters from the corresponding α-benzyloximino esters by treatment with $NaBH_3CN$ in acetic acid. The reaction involves initial reduction of the oxime double bond, followed by a reductive alkylation. Since, as far as we know, the synthesis of secondary N-hydroxy-α-amino acid derivatives by reductive alkylation has never been reported before, we decided to investigate the scope of this mild conversion in more detail.

Primarily our attention was focused on the reductive alkylation in carboxylic acid media. When no additional reagents are used, then the choice of the solvent determines the nature of the alkyl group. However, only a few carboxylic acids seem to be suitable as solvents, which limits the range of alkyl groups to be introduced in this way. It was realized that the addition of a large excess of an appropriate aldehyde to the reaction mixture might be a way to circumvent this restriction.

A study of the feasibility of this alternative approach was included in the investigation. The results are depicted in table 2.

Table 2



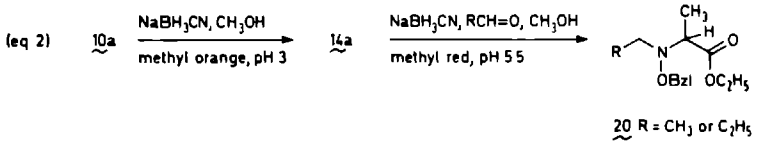
solvent	aldehyde (eq.)	eq.$NaBH_3CN$	yield(%)					
			4b	5b	8	17	18	19
HCOOH	-	3	-	15	-	68	-	-
HCOOH	$CH_3CH_2CH=O$ (21)	14	-	10	-	44	10	-
HCOOH	$C_6H_5CH=O$ (13)	10	50	-	-	18*	-	-
CH_3COOH	$CH_3CH_2CH=O$ (13)	10	-	10	23	-	34	-
CH_3COOH	$C_6H_5CH=O$ (13)	10	38	-	30*	-	-	-

* In these experiments also the formation of benzyl alcohol was observed.

The results show that the product formation cannot be controlled by the addition of a large amount of an aldehyde because of the interference by the solvent. Consequently the choice of another solvent had to be considered.

In preliminary experiments methanol was used as solvent for the

reductive alkylation of an N-benzyloxy-α-amino acid ester (eq.2: 14a → 20). Monitoring the reaction by TLC revealed that the fastest conversion of the starting material takes place at pH 5.0-5.5 ;



20 R = CH_3 or C_2H_5

methyl red was used to monitor the pH, which was held at the red-orange transition point (approximately pH 5.5) by the addition of 7N methanolic HCl. The results of an experiment carried out at this pH looked very promising, since according to TLC only one product had been formed.[9] Finally, it has been observed that a one-pot conversion of 10a to N-alkyl-N-benzyloxy amino acid ester 20 (eq.2) in MeOH is possible.

5.4 *CONCLUSIONS*

To our best knowledge this chapter presents the first examples of the reduction of α-(benzyl)oximino esters and amides with $NaBH_3CN$,[10] and of the synthesis of secondary N-hydroxy amino acid derivatives by reductive alkylation. As far as the reduction of α-(benzyl)oximino compounds is concerned, the synthetic usefulness of $NaBH_3CN$ is restricted to simple compounds. For the synthesis of compounds containing an amide function, *e.g.* N-hydroxy peptides, one has to fall back upon the amine-borane complexes.

On the other hand, the preliminary experiments described in section 5.3 indicate that for the reductive alkylation in an inert solvent like MeOH, $NaBH_3CN$ might be the reagent of choice. The

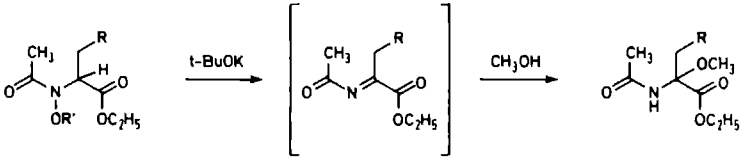
advantage of this way of introducing an alkyl group is that the chiral centre is not affected.

5.5 *APPENDIX*

The hydroxamic acids 7a-c have been used as model systems for studying the validity of an idea involving stereospecific conversion of N-hydroxy-α-amino acid derivatives into α-functionalized amino acid derivatives. This investigation was undertaken for the following reason.

In our laboratory a method for the synthesis of α-functionalized α-amino acid derivatives 23 has been developed, involving treatment of O-alkylated- or O-acylated N-acyl-N-hydroxy-α-amino acid derivatives 21 with a strong base in the presence of an excess of a nucleophile (eq.3).[1,11,12]

(eq 3)



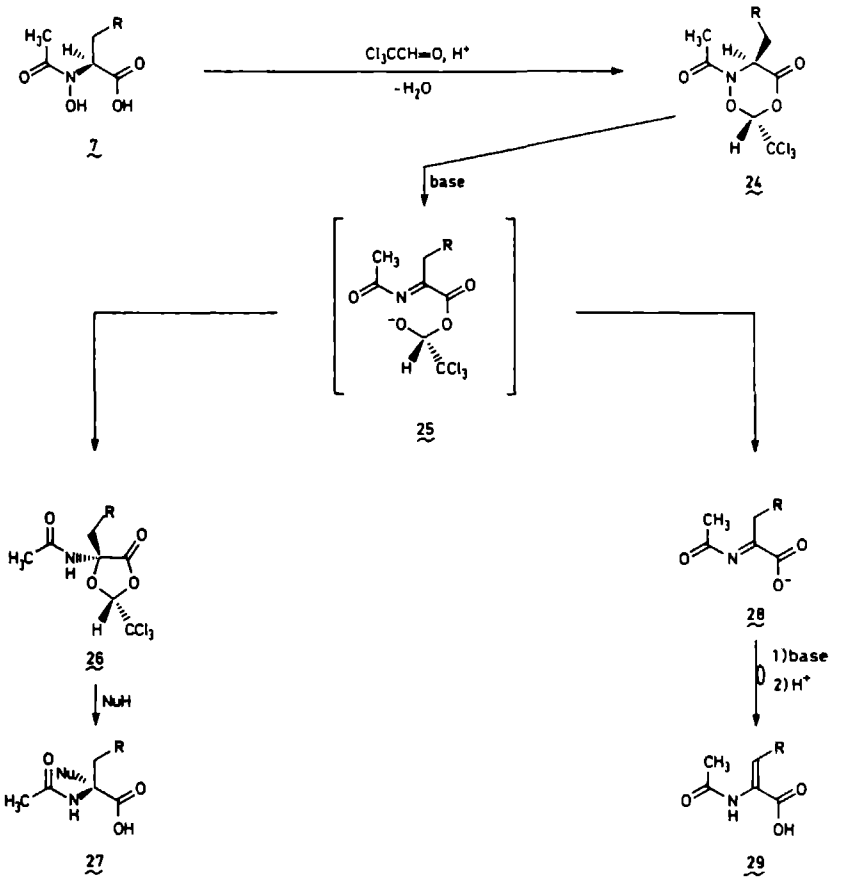
21 R' = Ac or Bzl 22 23

The intermediacy of an α-acylimino carboxylic acid derivative 22 was proposed.[1,11,12] Consequently, when no additional chiral centres are present in 21, the procedure is unsuited for the synthesis of optically active compounds.

We wondered whether it would be possible to accomplish such a transposition of an N-hydroxy group to an α-functionality in a stereospecific way by applying the so-called principle of self-reproduction of chirality, which has been formulated for the first

time by Seebach.[13] The framework of this idea is outlined in scheme III.

Scheme III



The first step should involve a diastereoselective condensation of an optically pure N-acetyl-N-hydroxy-α-amino acid 7 with chloral. Then a base-catalyzed rearrangement of 24 should afford the five-membered heterocycle 26 with inversion of configuration at the α-carbon atom. The intermediacy of 25 is obvious, and so the second chiral centre, created in the condensation step, has to preserve

the chirality at this stage; it must control the stereochemical course of the rearrangement. The chlorine atoms were expected to be helpful in stabilizing the negative charge on the oxygen atom in 25, thus preventing undesirable side reactions like the base-consuming formation of the α,β-dehydroamino acid derivative 29. The net result of the conversion should be a transposition of an N-hydroxy group to an α-functionality with retention of configuration (7 → 27).

To study the attainability of the chemical conversions we had in mind (scheme III), the optically inactive[14] N-acetyl-N-hydroxy-α-amino acids 7a-c were selected as model compounds. However, all attempts to accomplish the formation of 24 by refluxing a mixture of one of the hydroxamic acids 7a-c, chloral and a catalytic amount of trifluoro- or trichloroacetic acid in a variety of solvents, under azeotropic removal of water (if formed), failed.[15] In all cases the starting material was recovered unchanged. Further investigations had to be postponed in consequence of changed priorities.

5.6 *EXPERIMENTAL SECTION*

Melting points were taken on a Köfler hot stage (Leitz-Wetzlar) and are uncorrected. Infrared spectra were measured with a Perkin-Elmer Model 397 spectrophotometer. Proton magnetic resonance spectra were measured using a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer.

Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with an UV hand lamp, iodine vapour or Cl_2-TDM.[16] Hydroxamic acids were detected (red spots) by spraying with a 3% $FeCl_3$ (w/v) solution in concentrated HCl/MeOH (4:96, v/v).

For column chromatography Merck silica gel H (type 60) was used. The Miniprep LC (Jobin Yvon) was used for preparative HPLC.

α-Benzyloximino carboxylic acids 2

These compounds were prepared according to the procedure described by Herscheid *et. al.*[17]. For some data concerning *2a* and *2c*, see ref. 17. *2b* : solid; ^{1}H-NMR : δ 9.96 (s, 1H, CO_2H), 7.33 (s, 5H, C_6H_5), 5.26 (s, 2H, OCH_2), 2.60 (q, 2H, CH_2CH_3), 1.06 (t, 3H, CH_3).

Compound *2c* has also been prepared according to a slightly modified version of the published procedure.[17] To a vigorously stirred suspension of the sodium salt (13.8 g, 0.1 mol) of 3-methyl-2-oxo-butanoic acid *1c* and O-benzylhydroxylamine hydrochloride (16.75 g, 0.1 mol) in 550 ml of H_2O was added 10 ml of concentrated aqueous HCl (12N). Then the reaction mixture was heated gently (40-50°C) for about 15 minutes. While stirring was continued at room temperature for 3h, the product separated as an oil. When the oil had settled down the water layer was decanted and washed three times with EtOAc. EtOAc (250 ml) was added to the residue in the flask and the resulting solution was washed twice with H_2O. The combined EtOAc layers were dried over Na_2SO_4. Then the solvent was evaporated in vacuo to afford *2c* as an oil in quantitative yield. According to the ^{1}H-NMR spectrum only one isomer (Z or E) was obtained; ^{1}H-NMR : δ 9.35 (s, 1H, OH), 7.26 (s, 5H, C_6H_5), 5.21

(s, 2H, CH_2), 3.43 (m, 1H, CH), 1.23 (d, 6H, $(CH_3)_2$).

α-Benzyloximino 4-nitrophenyl esters 3

These compounds were also prepared according to a procedure described by Herscheid *et. al.*[17] For some data concerning *3a* and *3c*, see ref. 17. *3b* : 69% yield; m.p.: 79-81°C; ^{1}H-NMR : δ 8.22 and 7.28 (AA'BB', 4H, C_6H_4) 7.35 (s, 5H, C_6H_5), 5.35 (s, 2H, $NOCH_2$), 2.70 (q, 2H, $C\underline{H}_2CH_3$), 1.15 (t, 3H, CH_3).

α-Benzyloximino benzyl esters 4

A solution of DBU (9.12 g, 60 mmol) in 120 ml of CH_2Cl_2 was added dropwise to a stirred solution of *3* (60 mmol) and benzyl alcohol (6.48 g, 60 mmol) in 240 ml of CH_2Cl_2. Stirring was continued at room temperature until completion of the reaction (3h) as monitored by TLC (CH_2Cl_2/n-hexane, 1:1 v/v). The reaction mixture was washed successively with 0.1N aqueous HCl and 0.1N aqueous NaOH until the yellow colour of the organic layer had disappeared completely. The organic layer was then separated and dried (Na_2SO_4). The solvent was evaporated to give crude *4*, which could be used in the next reaction without further purification, in quantitative yield. Column chromatography on silica gel (Merck, silica gel H (type 60), CH_2Cl_2/n-hexane (1:1, v/v) as eluant) can be applied to remove traces of impurities. *4a* : oil; ^{1}H-NMR : δ 7.30 (s, 10H, $2xC_6H_5$), 5.25 (s, 4H, $2xOCH_2$), 2.04 (s, 3H, CH_3). *4b* : oil; ^{1}H-NMR : δ 7.40 (s, 10H, $2xC_6H_5$), 5.30 (s, 4H, $2xOCH_2$), 2.63 (q, 2H, $C\underline{H}_2CH_3$), 1.06 (t, 3H, CH_3). *4c* : oil; ^{1}H-NMR : δ 7.35 (s, 10H, 2x C_6H_5), 5.26 and 5.21 (2xs, 4H, $2xOCH_2$), 3.43 (m, 1H, CH), 1.16 (d, 6H, $(CH_3)_2$).

N-Benzyloxy-α-amino acid benzyl esters 5

Trimethylamine-borane (1.83 g, 25 mmol) was added at room temperature to a stirred solution of *4* (10 mmol) in 100 ml of anhydrous ether, saturated with dry HCl. Stirring was continued at room temperature until completion of the reaction (16h) as monitored by TLC (CH_2Cl_2). After evaporation of the solvent, the residue was dissolved in 50 ml of EtOAc. This solution was washed three times with 30 ml of a 5% aqueous $NaHCO_3$ solution and then once with brine. The organic layer was separated and dried (Na_2SO_4). The residue, obtained after evaporation of the solvent, was purified by column chromatography (Merck, silica gel H (type 60), diisopropyl ether/n-hexane (1:1, v/v) as eluant). *5a* : 45% yield[18]; oil; ^{1}H-NMR : δ 7.23 (s, 10H, 2xC_6H_5), 5.77 (br., 1H, NH), 5.13 (s, 2H, (O)$COCH_2$), 4.66 (s, 2H, $NOCH_2$), 3.73 (q, 1H, C$\underline{H}CH_3$), 1.17 (d, 3H, CH_3). *5b* : 58% yield; oil; ^{1}H-NMR : δ 7.33 and 7.30 (2xs, 10H, 2xC_6H_5), 5.98 (br., 1H, NH), 5.20 (s, 2H, (O)$COCH_2$), 4.71 (s, 2H, $NOCH_2$), 3.61 (m, 1H, CHN), 1.51 (m, 2H, C$\underline{H}_2CH_3$), 0.90 (t, 3H, CH_3). *5c* : 65% yield; oil; ^{1}H-NMR : δ 7.31 and 7.26 (2xs, 10H, 2xC_6H_5), 5.93 (br., 1H, NH), 5.20 (s, 2H, (O)$COCH_2$), 4.66 (s, 2H, $NOCH_2$), 3.45 (d, 1H, CHN), 2.13-1.46 (m, 1H, C$\underline{H}(CH_3)_2$), 0.93 and 0.81 (2xd, 6H, $(CH_3)_2$); exact mass calcd. for $C_{19}H_{24}NO_3$ (M^++1), m/e : 314.1756, found : 314.1758; chemical-ionization mass spectrum, m/e (relative intensity) : 314 ($[M+1]^+$, 8%), 181 ($[C_{14}H_{13}]^+$, 33%), 107 ($[C_7H_7O]^+$, 19%), 91 ($[C_7H_7]^+$, 13%).

N-Acetyl-N-benzyloxy-α-amino acid benzyl esters 6

A solution of pyridine (0.87 g, 11 mmol) in 5 ml of dry CH_2Cl_2 was added to an ice-cooled, stirred solution of *5* (10 mmol) and

freshly distilled acetyl chloride (0.86 g, 11 mmol) in 30 ml of dry CH_2Cl_2. Stirring was continued at room temperature for 1h after which time the salt was removed by filtration. The filtrate was washed with 0.1N aqueous HCl (2x30 ml), 5% aqueous $NaHCO_3$ (2x30 ml) and H_2O (1x30 ml). The organic layer was separated and dried (Na_2SO_4) and the solvent was evaporated to give pure *6*. *6a* : 94% yield; oil; ^{1}H-NMR : δ 7.31 and 7.26 (2xs, 10H, 2xC_6H_5), 5.13 (s, 2H, (O)$COCH_2$), 4.99 (q, 1H, $CHCH_3$), 4.84 (s, 2H, $NOCH_2$), 2.09 (s, 3H, $C(O)CH_3$), 1.53 (d, 3H, $CHCH_3$). *6b* : 93% yield; oil; ^{1}H-NMR : δ 7.33 (s, 10H, 2xC_6H_5), 5.18 (s, 2H, (O)$COCH_2$), 4.90 (t, 3H, $NOCH_2$ and CHN), 2.36-1.65 (m, 2H, CH_2CH_3), 2.13 (s, 3H, $C(O)CH_3$), 0.96 (t, 3H, CH_2CH_3). *6c* : 90% yield; oil; ^{1}H-NMR : δ 7.35 (s, 10H, 2x C_6H_5), 5.21 (s, 2H, (O)$COCH_2$), 4.96 and 4.80 (AB spectrum, J_{AB} = 11 Hz, 2H, $NOCH_2$), 4.79 (d, 1H, CHN), 2.90-2.11 (m, 1H, $CH(CH_3)_2$), 2.11 (s, 3H, $C(O)CH_3$), 1.03 and 0.90 (2xd, 6H, $(CH_3)_2$).

N-Acetyl-N-hydroxy-α-amino acids 7

A solution of *6* (10 mmol) in 100 ml of MeOH was treated at room temperature and atmospheric pressure with H_2 and 10% Pd/C (150 mg) until 450 ml of H_2 (20 mmol) had been consumed. After removal of the catalyst by filtration the solvent was evaporated to give pure *7* in quantitative yield. The compound was crystallized from acetone/n-hexane. *7a* : oil; ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1 v/v): δ 5.07 (q, 1H, $CHCH_3$), 2.16 (s, 3H, $C(O)CH_3$), 1.44 (d, 3H, $CHCH_3$). *7b* : m.p. 112-114°C; ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1 v/v): δ 5.41-4.68 (m, CHN and HDO[19]), 2.21 (s, 3H, $C(O)CH_3$), 2.21-1.60 (m, 2H, CH_2CH_3), 0.95 (t, 3H, CH_2CH_3); IR (KBr) : 3360, 3100-2200, 1720 and 1605 cm^{-1}; exact mass calcd. for $C_6H_{12}NO_4$ (M^++1), m/e 162.0766, found: 162.0767; chemical-ionization mass spectrum, m/e (relative

intensity): 162 ($[M+1]^+$, 19%), 146 ($[M+1-O]^+$, 36%), 128 ($[M+1-O-H_2O]^+$, 10%), 120 ($[M+1-CH_2CO]^+$, 26%), 104 ($[M+1-O-CH_2CO]^+$, 45%), 100 ($[M+1-H_2O-CO_2]^+$, 59%), 74 ($[CH_3CH_2CH(=NOH)+1]^+$, 25%), 58 ($[CH_3CH_2CH(=NH)+1]^+$, 100%); anal. calcd. for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69, found: C, 44.65; H, 6.87; N, 8.66. *7c* : m.p. 139-141°C; ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1 v/v): δ 4.86 (d, 1H, CHN), 2.83-1.75 (m, 1H, C<u>H</u>$(CH_3)_2$), 2.18 (s, 3H, $C(O)CH_3$), 1.11 and 0.90 (2xd, 6H, $(CH_3)_2$); IR (KBr): 3380, 3100-2200, 1710 and 1605 cm^{-1}; exact mass calcd. for $C_7H_{14}NO_4$ (M^++1), m/e 176.0923, found: 176.0920; chemical-ionization mass spectrum, m/e (relative intensity): 176 ($[M+1]^+$, 27%), 160 ($[M+1-O]^+$, 52%), 142 ($[M+1-O-H_2O]^+$, 16%), 134 ($[M+1-CH_2CO]^+$, 19%), 118 ($[M+1-O-CH_2CO]^+$, 31%), 114 ($[M+1-H_2O-CO_2]^+$, 100%), 88 ($[(CH_3)_2CHCH(=NOH)+1]^+$, 21%), 72 ($[(CH_3)_2CHCH(=NH)+1]^+$, 79%); anal. calcd. for $C_7H_{13}NO_4$: C, 47.99; H, 7.48; N, 8.00, found: C, 47.74; H, 7.47; N, 7.95.

Attempted conversion of <u>4b</u> into <u>6b</u> with $NaBH_3CN$/acetic anhydride

$NaBH_3CN$ (0.28 g, 4.4 mmol) and acetic anhydride (3.14 g, 30.8 mmol) were added at room temperature to a stirred solution of *<u>4b</u>* (1.30 g, 4.4 mmol) in 20 ml of acetic acid. After stirring for three days the conversion was not complete as judged by TLC (CH_2Cl_2). After adding an additional amount of $NaBH_3CN$ (0.28 g, 4.4 mmol) and acetic anhydride (2.24 g, 22 mmol) to the reaction mixture stirring was continued at room temperature for another 4 days. Then the reaction was interrupted although it was still not complete as monitored by TLC (CH_2Cl_2). The solvent was evaporated in vacuo, and the resulting residue was dissolved in Et_2O. The solution was washed with 5% (w/v) aqueous $NaHCO_3$ (3x) and brine, dried over Na_2SO_4 and finally concentrated to dryness in vacuo. The

residue was purified by column chromatography (solvent system: CH_2Cl_2/n-hexane, 3:1 v/v), affording the starting material *4b* (44%), the desired product *6b* (36%) and *benzyl N-ethyl-N-benzyloxy-α-aminobutanoate 8* as an oil in 20% yield; ^{1}H-NMR : δ 7.30 and 7.25 (2xs, 10H, 2xC_6H_5), 5.16 (s, 2H, (O)$COCH_2$), 4.72 (s, 2H, $NOCH_2$), 3.50 (dd, 1H, C(O)CH), 3.13-2.70 (m, 2H, NCH_2), 1.96-1.61 (m, 2H, $CHCH_2CH_3$), 1.18 (t, 3H, NCH_2CH_3), 0.90 (t, 3H, $CHCH_2CH_3$); exact mass calcd. for $C_{20}H_{26}NO_3$ (M^++1), m/e 328.1913, found: 328.1920; chemical-ionization mass spectrum, m/e (relative intensity): 328 ($[M+1]^+$, 100%), 236 ($[M-C_7H_7]^+$, 8%), 192 ($[M-C(O)OCH_2C_6H_5]^+$, 29%), 176 ($[M-CO_2-OCH_2C_6H_5]^+$, 6%), 133 (6%), 107 ($[C_7H_7O]^+$, 8%), 91 ($[C_7H_7]^+$, 54%).

The preparation of the α-(benzyl)oximino acid derivatives *9-12* has been described previously.[2b] Only the synthesis of *9f* and *10f* differed from this procedure:

Ethyl 3-phenyl-2-oximino-propanoate 9f.

To a solution of phenylpyruvic acid sodium salt monohydrate (6.12 g, 30 mmol) in 50 ml of EtOH 10 ml of 7N ethanolic HCl was added. After stirring at room temperature for one hour, TosOH (150 mg), hydroxylamine hydrochloride (2.09 g, 30 mmol) and EtOH (150 ml) were added. The resulting mixture was heated under reflux to remove water azeotropically. Meanwhile an additional amount of EtOH (150 ml) was added dropwise to the reaction mixture. After 2 hours of reflux 250 ml of distillate was collected and the reaction was complete as monitored by TLC (solvent system : CH_2Cl_2). Then the mixture was concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, washed with 0.05N aqueous NaOH and brine, and dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded

crude *9f*, which was subjected to column chromatography (solvent system : MeOH/CH_2Cl_2, 1:99 v/v). Pure *9f* was thus obtained in 79% yield; ^{1}H-NMR : δ 10.20 (br., 1H, N-OH), 7.26 (m, 5H, C_6H_5), 4.29 (q, 2H, $C\underline{H}_2CH_3$), 4.00 (s, 2H, $C_6H_5C\underline{H}_2$), 1.30 (t, 3H, CH_3); chemical-ionization mass spectrum, m/e (relative intensity) : 208 ($[M+1]^+$, 100%), 190 ($[M-OH]^+$, 21%), 162 ($[M-OC_2H_5]^+$, 98%), 134 ($[M-CO_2C_2H_5]^+$, 14%), 118 ($[C_6H_5CH_2CN+1]^+$, 70%), 91 ($[C_7H_7]^+$, 67%).

Ethyl 3-phenyl-2-benzyloximino-propanoate 10f

The compound was prepared according to the procedure described above, using O-benzylhydroxylamine hydrochloride (4.79 g, 30 mmol) instead of hydroxylamine hydrochloride. The crude product, obtained after concentration of the reaction mixture in vacuo, was dissolved in CH_2Cl_2, washed with 0.1N aqueous HCl and 0.1N aqueous NaOH, and dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded pure *10f* in 86% yield; ^{1}H-NMR : δ 7.30 and 7.18 (2xs, 10H, 2xC_6H_5), 5.31 (s, 2H, $C_6H_5C\underline{H}_2O$), 4.26 (q, 2H, $C\underline{H}_2CH_3$), 3.95 (s, 2H, $C_6H_5C\underline{H}_2C$), 1.28 (t, 3H, CH_3); exact mass calcd. for $C_{18}H_{20}NO_3$ (M^++1), m/e : 298.1443, found : 298.1435; chemical-ionization mass spectrum, m/e (relative intensity) : 298 ($[M+1]^+$, 21%), 252 ($[M-OC_2H_5]^+$, 12%), 190 ($[M-C_7H_7O]^+$, 6%), 118 ($[C_6H_5CH_2CN+1]^+$, 26%), 107 ($[C_7H_7O]^+$, 7%), 91 ($[C_7H_7]^+$, 100%).

General procedure for the reduction of α-(benzyl)oximino esters and amides with $NaBH_3CN$

With regular intervals of at least one hour $NaBH_3CN$ was added in portions of 0.32 g (5 mmol) to a stirred solution of the α-oximino acid derivative (5 mmol) and a trace of methyl orange in 10 ml of MeOH at room temperature. During the reaction the pH was

held at the red-orange transition point of the indicator by the dropwise addition of 7N methanolic HCl. The progress of the reaction was monitored by TLC, using CH_2Cl_2 or a MeOH/CH_2Cl_2-mixture as eluant. In general the reaction mixture was stirred at room temperature for 2-3 days. The reaction was interrupted by neutralizing the reaction mixture with $NaHCO_3$. After filtration the solvent was evaporated in vacuo. The residue was dissolved in a mixture of EtOAc (20 ml) and 0.5M aqueous $NaHCO_3$ (20 ml). Then the layers were separated and the water layer was washed with EtOAc (20 ml). The organic layers were combined, washed with brine, dried over Na_2SO_4 and finally concentrated to dryness in vacuo. The yield of *5* and *13-16* (Table 1) was determined from the ^{1}H-NMR spectrum of the crude product. The product could be separated from the starting material by column chromatography, using CH_2Cl_2 or a MeOH/CH_2Cl_2-mixture as eluant. The spectroscopic data of the pure products have been reported before.[2b,c]

General procedure for the reductive alkylation in carboxylic acids

With regular intervals of at least one hour $NaBH_3CN$ was added in portions of 0.113 g (1.8 mmol) to a solution of *4b* (0.53 g, 1.8 mmol) and, if relevant, the appropriate aldehyde (13-21 eq.) in 10 ml of formic or acetic acid at room temperature. The reaction mixture was stirred at room temperature for 1-2 days. The carboxylic acid was evaporated in vacuo and the residue was dissolved in Et_2O. The solution was washed with 5% (w/v) aqueous $NaHCO_3$ (3x) and brine, dried over Na_2SO_4 and finally concentrated to dryness in vacuo. The residue was subjected to column chromatography, using CH_2Cl_2/n-hexane (1:1, v/v) as eluant. The spectroscopic data of the products are as follows : *8*, *benzyl N-ethyl-N-benzyloxy-α-amino-*

butanoate : vide supra. 17. benzyl N-methyl-N-benzyloxy-α-amino-butanoate; ^{1}H-NMR : δ 7.29 and 7.24 (2xs, 10H, 2xC_6H_5), 5.16 (s, 2H, CO_2CH_2), 4.66 (s, 2H, $NOCH_2$), 3.36 (dd, 1H, $CHCO_2$), 2.64 (s, 3H, NCH_3), 1.93-1.49 (m, 2H, CH_2CH_3), 0.89 (t, 3H, CH_2CH_3); exact mass calcd. for $C_{19}H_{24}NO_3$ (M^++1), m/e : 314.1756, found : 314.1749; chemical-ionization mass spectrum, m/e (relative intensity) : 314 ($[M+1]^+$, 100%), 222 ($[M-C_7H_7]^+$, 2%), 206 ($[M-C_7H_7O]^+$, 4%), 178 ($[M-CO_2CH_2C_6H_5]^+$, 54%), 162 ($[M-CO_2-OCH_2C_6H_5]^+$, 15%), 133 (6%), 107 ($[C_7H_7O]^+$, 4%), 91 ($[C_7H_7]^+$, 53%). *18. benzyl N-(n-propyl)-N-benzyloxy-α-aminobutanoate*; ^{1}H-NMR : δ 7.29 and 7.24 (2xs, 10H, 2x C_6H_5), 5.14 (s, 2H, CO_2CH_2), 4.71 (s, 2H, $NOCH_2$), 3.50 (dd, 1H, $CHCO_2$), 2.95-2.54 (m, 2H, CH_2N), 1.98-1.43 (m, 4H, $NCH_2CH_2CH_3$ and $CHCH_2CH_3$), 0.91 (t, 6H, 2xCH_2CH_3); exact mass calcd. for $C_{21}H_{28}NO_3$ (M^++1), m/e : 342.2069, found : 342.2067; chemical-ionization mass spectrum, m/e (relative intensity) : 342 ($[M+1]^+$, 43%), 250 ($[M-C_7H_7]^+$, 9%), 234 ($[M-C_7H_7O]^+$, 8%), 206 ($[M-CO_2CH_2C_6H_5]^+$, 21%), 190 ($[M-CO_2-OCH_2C_6H_5]^+$, 4%), 107 ($[C_7H_7O]^+$, 7%), 91 ($[C_7H_7]^+$, 100%).

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oximino compounds, mild reagents have to be employed to avoid overreduction to the corresponding α-amino acid derivatives. Amine-borane complexes and cyanoborohydrides meet this requirement. Whereas amine-borane complexes are generally applicable, it has been reported that reduction with $NaBH_3CN$ is limited to α-oximino acids : see ref. 2a and 2b.

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8. When bromocresol green was used as indicator and the pH was held at the yellow-green transition point ("pH 4"), the reduction rate was not sufficiently rapid to be synthetically useful.
9. The product has not been isolated. Another experiment, carried out at pH 6-7 and using propanal as the aldehyde, afforded in addition to recovered starting material (14a : 82%) 20 (R=CH_2CH_3) in 17% yield. The spectroscopic data of 20 (R=CH_2CH_3) are as follows : ^{1}H-NMR ($CDCl_3$) : δ 7.19 (s, 5H, C_6H_5), 4.67 (s, 2H, $NOCH_2$), 4.10 (q, 2H, OCH_2CH_3), 3.51 (q, 1H, $CHCO_2$), 2.95-2.44 (m, 2H, NCH_2), 1.81-1.40 (m, 2H, $CH_2CH_2CH_3$), 1.25 (d, 3H, $CHCH_3$), 1.19 (t, 3H, OCH_2CH_3), 0.85 (t, 3H, $CH_2CH_2CH_3$); exact mass calcd. for $C_{15}H_{24}NO_3$ (M^++1), m/e : 266.1756, found : 266.1759; chemical-ionization mass spectrum, m/e (relative intensity) : 266 ($[M+1]^+$, 100%), 192 ($[M-CO_2C_2H_5]^+$, 63%), 174 ($[M-C_6H_5CH_2]^+$, 28%), 158 ($[M-C_6H_5CH_2O]^+$, 22%), 91 ($[C_7H_7]^+$, 49%).
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15. a) Similarly, when using pivalaldehyde instead of chloral there was no formation of any product. b) A condensation of L-proline with chloral in refluxing n-pentane in the presence of a catalytic amount of trifluoroacetic acid, with azeotropic removal of the water formed, proceeded smoothly, probably furnishing just one diastereomer. Some time later the same reaction was reported in the literature: Polonski, T., Tetrahedron (1985), *41*, 603.
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18. In this case the rather low yield was caused by an inefficient separation of the product from the trimethylamine-borane complexes by column chromatography.
19. CD_3OD was contaminated with H_2O.

Hydroxamzuren afgeleid van N-hydroxy-α-aminozuren: syntheses en toepassingen

Hydroxamzuren zijn verbindingen die veelvuldig in de natuur aangetroffen worden; zij worden gekenmerkt door de aanwezigheid van een geoxideerde amide band, de zogenaamde hydroxamzuur funktie (1, p.2). De eigenschappen van deze verbindingen worden in belangrijke mate bepaald door deze funktie. Het zijn zwakke zuren met een pK_a-waarde die, afhankelijk van het substitutie patroon, varieert van 7 tot 11. Een andere in het oog springende eigenschap is het vermogen om zeer stabiele complexen te vormen met metaalionen, met name met ionen van overgangsmetalen (schema III, p.11). De hoogste complexatie constanten worden vertoond door de intens roodgekleurde ferri-complexen (schema V, p.12). Het is derhalve niet verbazingwekkend dat veel lagere organismen juist hydroxamzuren gebruiken voor het verzamelen en vasthouden van metaalionen.

De meest voor de hand liggende manieren waarop hydroxamzuren gevormd kunnen worden, zijn oxidatie van een amide band en N-acylering van een hydroxylamine derivaat (schema XVII,p.29). Aangetoond is dat beide processen in de natuur plaatsvinden.

In dit proefschrift vormen hydroxamzuren die afgeleid zijn van N-hydroxy-α-aminozuren, d.w.z. N-acyl-N-hydroxy-α-aminozuur derivaten en N-hydroxy peptides (5, p.3), het centrale thema. De probleemstelling heeft betrekking op de synthese, toepassing en biosynthetische relevantie van deze klasse van chirale hydroxamzuren.

Hoofdstuk I is een algemene inleiding waarin onder meer de werkhypothese geschetst wordt die ten grondslag ligt aan een belangrijk deel van het onderzoek dat in dit proefschrift beschreven wordt. Volgens deze werkhypothese, die voortkomt uit het in de jaren 1978-1982 in de werkgroep verrichte onderzoek, kan de verscheidenheid aan gemodificeerde aminozuren, die kenmerkend is voor een groot aantal schimmelmetabolieten, o.a. via N-hydroxy-α-aminozuur derivaten ontstaan.

De N-hydroxy-α-aminozuur derivaten nemen in de werkhypothese zowel in de onderlinge relaties tussen gemodificeerde aminozuren als in de samenhang tussen gewone en gemodificeerde aminozuren een centrale plaats in (schema I, p.3). Gepostuleerd werd dat deze schematische samenhang van zowel synthestische als biosynthetische betekenis is.

Diverse aspecten van de chemie van hydroxamzuren worden nader toegelicht in hoofdstuk II. Onder andere wordt aandacht geschonken aan de aciditeit, de (bio-)synthese en de reactiviteit van hydroxamzuren, alsmede aan de complexatie met metaalionen.

In hoofdstuk III wordt een onderzoek naar de toepasbaarheid van chirale hydroxamzuren als liganden in asymmetrische, metaalgekatalyseerde reacties beschreven. Hydroxamzuren zijn in dit opzicht interessante verbindingen gezien hun vermogen om met verschillende metaalionen stabiele complexen te vormen; derhalve zijn ze potentieel bruikbaar zijn in allerlei metaal-gekatalyseerde reacties.

In het kader van dit onderzoek werden een aantal rigide hydroxamzuren gesynthetiseerd uitgaande van goedkope uitgangsstoffen, nl. (S)-proline en enige racemische α-halocarbonzuren (schema III, p.41). Als intermediairen werden de N-(2-bromo-acyl)-(S)-

proline derivaten 10-13 gesynthetiseerd in opbrengsten variërend van 54% tot 86%. In de meeste gevallen (11, 12 en 13) werd een mengsel van diastereomeren verkregen. Bovendien bleek dat m.b.v. ^{1}H-NMR spectroscopie in oplossing rotameren van de verbindingen 12b, 13a en 13b gedetecteerd kunnen worden (fig.1, p.42). Verbinding 13, die een aantal malen in 88% opbrengst gesynthetiseerd werd uit het van 9 afgeleide zuurbromide (schema IV, p.43), vertoonde een bijzondere eigenschap. Terwijl chromatografische scheiding van de diastereomeren 13a en 13b niet mogelijk was, kon het diastereomerenmengsel quantitatief omgezet worden in 13a d.m.v. een kristallisatie-geïnduceerde asymmetrische transformatie. Dissociatie van de C-Br band ligt waarschijnlijk ten grondslag aan dit proces.

Na behandeling van de verbindingen 10, 12a en 13a met hydroxylamine werden de gewenste hydroxamzuren 14, 16a en 17a in resp. 25%, 61% en 75% opbrengst geïsoleerd. Op dezelfde wijze kon uit het diastereomerenmengsel 11a,b uiteindelijk slechts één zuiver diastereomeer (15b) verkregen worden in 46% opbrengst. Ringsluiting van 12b leverde in 20% opbrengst een optisch actief mengsel van beide enantiomeren van 16a op (schema V, p.47). Verbinding 17b epimeriseerde onder de reactieomstandigheden tot 17a (eq.4, p.48) en kon in slechts 14% opbrengst uit het chloor-analogon van 13b (21b, p.46) verkregen worden.

Het chiraal inducerende vermogen van de hydroxamzuren werd getoetst in een bekende metaalgekatalyseerde reaktie, nl. de epoxidatie van allylalcoholen (Sharpless-epoxidatie). De beste resultaten werden behaald met een katalytische hoeveelheid van een vanadiumcomplex van 17a; afhankelijk van de omstandigheden

(temperatuur, aantal equivalenten hydroxamzuur) en het gebruikte substraat werden optisch actieve epoxyalcoholen verkregen met een enantiomere overmaat van 38-66% (eq.7 en tabel 1, p.51; tabel 2, p.53). Toepassing van andere overgangsmetalen (titanium, molybdeen) leverde slechtere resultaten op (eq.8 en tabel 3, p.53,54).

Vervolgens werd de invloed van twee hydroxamzuren op het stereochemisch verloop van een andere metaal-gekatalyseerde reaktie, nl. de oxidatie van een sulfide, bestudeerd. Gebruik makend van de katalysator, die in de Sharpless-epoxidatie de beste resultaten had opgeleverd, d.w.z. 1% V^{5+} op molbasis en 3 of 5% 17a, werd in de oxidatie van thioanisol het betreffende sulfoxide verkregen met een optische zuiverheid van 6.5 tot 11% (eq.9 en tabel 4, p.54,55). Toepassing van 17b in deze reaktie leidde tot een sulfoxide met een optische zuiverheid van slechts 3%.

De resultaten wijzen erop dat, bij aanwezigheid van een substituent op de 6-positie (zie 1, p.39) in een trans-oriëntatie t.o.v. de proline ring, beide chirale centra een coöperatief effect hebben op de chirale inductie.

Hoofdstuk IV is gewijd aan de synthese van een aantal N-hydroxy peptides (35-37, p.105) in het kader van een onderzoek naar de biosynthese van penicilline.

In de biosynthese van penicilline (schema I, p.94) funktioneert het zogenaamde Arnstein tripeptide (14, p.94) als precursor van isopenicilline N (15, p.94). Ondanks naarstig onderzoek was, toen wij met dit onderzoek begonnen, nog niet vastgesteld via welk mechanisme 14 door het enzym isopenicilline N synthetase in het antibioticum 15 omgezet wordt. Het bicyclische skelet van penicilline is opgebouwd uit twee β-gefunktionaliseerde α-aminozuren (cysteïne

en valine). Op basis van onze werkhypothese (schema I, p.3) hebben wij gepostuleerd dat de N-hydroxy peptides 35-37 (p.105) als intermediairen optreden in de omzetting van 14 in 15 (schema IX, p.107; schema X, p.108). Het was de bedoeling dit postulaat te toetsen door incubatie van elk der N-hydroxy tripeptides 35-37 met het isopenicilline N synthetase.

Met dit oogmerk werd aanvankelijk gelijktijdig aan de synthese van de drie verschillende N-hydroxy tripeptides 35-37 gewerkt. De synthese van 35 en 36 werd echter niet voltooid omdat gaandeweg publicaties verschenen, waaruit bleek dat beide verbindingen geen rol spelen in de biosynthese van penicilline. Overigens dient vermeld te worden dat een cruciale stap in de bereiding van 35 (schema XI, p.112), namelijk de koppeling van een cysteïne derivaat (44) met racemisch N-benzyloxy valine benzyl ester 45, gerealiseerd kon worden m.b.v. fosgeen als activerend reagens. Het beschermde N-hydroxy dipeptide 46 werd als een mengsel van diastereomeren in 25% opbrengst verkregen.

De synthese van 37, evenals 36 (schema XX, p.127) een N-hydroxy cysteïne derivaat, werd wel afgerond. Voor N-hydroxy cysteïne derivaten zijn in de literatuur geen precedenten voorhanden. Ze zijn niet toegankelijk via directe oxidatie van een cysteïne derivaat vanwege de aanwezigheid van het gemakkelijk oxideerbare zwavelatoom. De gekozen benadering (schema XXV, p.135) behelsde de bereiding van drie N-hydroxy dipeptide derivaten (111-113, p.135) uit een β-bromo-α-oximino derivaat door nucleofiele substitutie met drie verschillende mercaptanen, gevolgd door selectieve reduktie van de oxim C=N band. Met behulp van deze procedure werden de beschermde N-hydroxy dipeptides 111-113 (p.135) in 13-38% opbrengst

verkregen uit 105. De volgende sleutelreactie was de koppeling van deze verbindingen met een beschermd α-aminoadipinezuur derivaat. In verband met de noodzakelijk sterke activering van de γ-carbonzuur funktie (eq.9, p.111) was een wijziging nodig van de aanvankelijk gekozen beschermgroepen strategie ten aanzien van α-aminoadipinezuur (schema XXVII, p.140). Uit een modelexperiment was namelijk gebleken dat een intramoleculaire reaktie van het urethaan N-atoom met het zuurchloride kan optreden (eq.24, p.141). De α-aminogroep van 126 (schema XXVIII, p.143) moest daarom zodanig beschermd worden dat het stikstofatoom niet meer nucleofiel was; dit werd gecombineerd met de bescherming van de α-carbonzuur funktie. Koppeling van 112 en 113 met het geactiveerde α-aminoadipinezuur derivaat 136 (eq.27, p.144) leverde de volledig beschermde N-hydroxy tripeptides 138 en 139 op in resp. 81% en 58% opbrengst.

Voor de ontschermingsreakties waren twee zuivere diastereomeren van 138 en één zuiver diastereomeer van 139 beschikbaar, allen met een onbekende configuratie van het chirale cysteïne koolstofatoom. Afhankelijk van de aard van de S-beschermgroep werden verschillende ontschermingsmethoden bestudeerd (schema XXV, p.135 ; schema XXIX, p.147). Alhoewel in tegenstelling tot de overige stappen in de synthese deze reakties slecht verliepen, lukte het uiteindelijk toch om in lage opbrengst een zuiver N-hydroxy tripeptide te synthetiseren uit 139 m.b.v $B(O_2CCF_3)_3$.

Incubatie van het verkregen N-hydroxy tripeptide met isopenicilline N synthetase leverde een biologisch actief produkt op waarvan de structuur vooralsnog onbekend is; waarschijnlijk hebben we te maken met N-hydroxy isopenicilline N (145, p. 151). Uit dit resultaat moet voorlopig geconcludeerd worden dat het postulaat dat

ten grondslag lag aan dit onderzoek in elk geval niet opgaat voor de biosynthese van penicilline.

Tenslotte worden in hoofdstuk V de eerste voorbeelden gegeven van de bereiding van primaire en secundaire N-hydroxy-α-aminozuur derivaten uit de overeenkomstige α-oximinozuur derivaten m.b.v. $NaBH_3CN$. De reduktie van α-oximinozuur derivaten, een sleutelstap in de bereiding van diverse in dit proefschrift beschreven hydroxamzuren, kan met $NaBH_3CN$ onder milde omstandigheden uitgevoerd worden (schema II, p.203). In het algemeen worden met deze methode echter lagere opbrengsten gerealiseerd dan bij toepassing van de tot dusverre gebruikte reductiemiddelen, namelijk pyridine- of trimethylamine-boraan in sterk zuur milieu (tabel 1, p.204). Voor een analoge omzetting in de bereiding van N-hydroxy peptides is $NaBH_3CN$ echter niet geschikt.

In methanol kunnen N-hydroxy-α-aminozuur derivaten met behulp van een aldehyde en $NaBH_3CN$ reductief gealkyleerd worden tot de overeenkomstige secundaire N-hydroxy-α-aminozuur derivaten (eq.2, p.207). Als voor deze reactie een carbonzuur als oplosmiddel gebruikt wordt, verloopt de alkylering ten gevolge van oplosmiddelparticipatie niet meer eenduidig (tabel 2, p.206).

Tenslotte werd vastgesteld dat in aanwezigheid van een aldehyde een één-pots omzetting van een α-oximinozuur derivaat in een secundair N-hydroxy-α-aminozuur derivaat mogelijk is met behulp van $NaBH_3CN$ (eq. 2, p.207).

1. Rob M.J. Liskamp, Hubertus J.M. Zeegers and Harry C.J. Ottenheijm.
   Synthesis and Ring-opening Reactions of Functionalized Sultines. A New Approach to Sparsomycin. *J.Org.Chem.* (1981), *46*, 5408.

2. J.M.M. Smits, P.T. Beurskens, B. Zeegers and H.C.J. Ottenheijm.
   Crystal and molecular structure of N-(S)-α-bromophenylacetyl-(S)-proline methyl ester, $C_{14}H_{16}NO_3Br$. *J. Crystallogr. Spectrosc. Res.* (1986), *16*, 739.

3. J.M.M. Smits, P.T. Beurskens, B. Zeegers and H.C.J. Ottenheijm.
   Crystal and molecular structure of cyclo-(S-prolyl-R-N-hydroxy-phenylglycyl), $C_{13}H_{14}N_2O_3$. *J. Crystallogr. Spectrosc. Res.* (1986) *16*, 747.

4. V. Busetti, H.C.J. Ottenheijm, H.J.M. Zeegers, D. Ajo and M. Casarin.
   Crystal and molecular structure of N-acetyl-N-hydroxy-α-amino acids. *Recl. Trav. Chim. Pays-Bas* (1987), *106*, 151.

5. H.J.M. Zeegers, P.T. Beurskens, W. Boonman, R.J.F. Nivard and H.C.J. Ottenheijm.
   N-Hydroxy-2,5-dioxopiperazines Derived from Proline: Chiral Hydroxamic Acids as Ligands for Asymmetric, Metal-catalyzed Reactions. Manuscript in preparation.

6. H.J.M. Zeegers, E.O.M. Orlemans, H.E.J. Hendriks, J.D.M Herscheid, G.I. Tesser, H.C.J. Ottenheijm, R.M. Adlington and J.E. Baldwin.
   Synthesis of N-[δ-(L-α-aminoadipyl)]-N-hydroxy-L-cysteinyl-D-valine: a Proposed Intermediate in the Biosynthesis of Penicillin. Manuscript in preparation.

Abstracts of papers presented at international meetings

1. H.J.M. Zeegers, E.O.M. Orlemans, H.E.J. Hendriks, J.D.M. Herscheid and H.C.J. Ottenheijm.
   Approaches to an N-Hydroxy Arnstein Tripeptide: a Possible Intermediate in the Biosynthesis of Penicillin.

   1st Belgian Organic Synthesis Symposium (BOSS-1), Namur, Belgium, 1986; 12th International Symposium on the Organic Chemistry of Sulfur, Nijmegen, the Netherlands, 1986.

2. H.J.M. Zeegers and H.C.J. Ottenheijm.
   Chiral Hydroxamic Acids as Ligands in Asymmetric, Metal-catalyzed Reactions.

   15th IUPAC International Symposium on the Chemistry of Natural Products, the Hague, the Netherlands, 1986.

Ac	acetyl
7-ACA	7-aminocephalosporanic acid
AcOH	acetic acid
(LLD)-ACV	δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine
6-APA	6-aminopenicillanic acid
BOC,Boc	t-butyloxycarbonyl
$(n\text{-}Bu)_3N$	tri-n-butylamine
n-BuOH	n-butanol
t-BuOK	potassium t-butoxide
Bzl	benzyl
BzlCl	benzyl chloride
BzlOH	benzyl alcohol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCHA	dicyclohexylamine
DIBAL-H	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	N,N-dimethylformamide
DTT	dithiothreitol
ENZ	enzyme
Et	ethyl
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
IPN	isopenicillin N
IPNS	isopenicillin N synthetase
MBOC	4-methoxybenzyloxycarbonyl
MCPBA	3-chloroperoxybenzoic acid
Me	methyl
MeOH	methanol
NMP	N-methylpiperidine
Nps	2-nitrophenylsulfenyl
ONP	4-nitrophenyloxy
Pd(C)	palladium on activated carbon (palladium content 10%)
Ph	phenyl
PMB	4-methoxybenzyl
PNB	4-nitrobenzyl
TBHP	t-butyl hydroperoxide
TDM	4,4'-tetramethyldiaminodiphenylmethane
TEA	triethylamine
THF	tetrahydrofuran
TMA	trimethylamine
TosCl	4-toluenesulfonyl chloride
$TosOH(.H_2O)$	4-toluenesulfonic acid (monohydrate)
Z	benzyloxycarbonyl

Bert Zeegers werd op 25 september 1957 geboren te Stein. In juni 1975 behaalde hij het diploma Atheneum-B aan de Albert Schweitzer Scholengemeenschap te Geleen. In september van hetzelfde jaar begon hij met de studie scheikunde aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen (S2) werd afgelegd in december 1978. De doctoraalstudie omvatte als eerste hoofdvak Organische Chemie (Prof. Dr. R.J.F. Nivard, Dr. H.C.J. Ottenheijm), als tweede hoofdvak Biochemie (Prof. Dr. H. Bloemendal, Prof. Dr. H.P.J. Bloemers), en werd aangevuld met een caputcollege Chemische Technologie (Prof. Dr. C. van Heerden). Het doctoraalexamen werd in juni 1982 afgelegd.

Van 1 juli 1982 tot 1 juli 1986 was hij, in dienst van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), als adjunct-wetenschappelijk medewerker verbonden aan het Laboratorium voor Organische Chemie van de Katholieke Universiteit te Nijmegen. In deze periode werd onder leiding van Dr. H.C.J. Ottenheijm en Prof. Dr. R.J.F. Nivard het in dit proefschrift beschreven onderzoek uitgevoerd. In augustus - september 1986 bracht hij een werkbezoek aan het Dyson Perrins Laboratorium (Prof.Dr. J.E. Baldwin) van de Universiteit van Oxford, daartoe in de gelegenheid gesteld door financiële steun van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO).

Gedurende zijn studie en zijn promotieonderzoek is hij als practicumassistent betrokken geweest bij het onderwijs aan studenten.

Sinds 1 januari 1987 is hij werkzaam bij Andeno B.V. te Venlo.

1. Het feit dat alleen het R-enantiomeer verantwoordelijk is voor de langdurige opname van ibuprofeen in vetweefsel en derhalve op langere termijn mogelijk bijwerkingen kan veroorzaken, toont opnieuw aan dat bij de toepassing van een racemisch geneesmiddel het onwerkzame enantiomeer - in dit geval R-ibuprofeen - niet zonder meer als onschadelijke ballast bestempeld mag worden.

   Williams, K; Day, R.; Knihinicki, R.; Duffield, A., *Biochem. Pharmacol.* (1986), *35*, 3403.

2. De vorming van een spoortje 4-nitrobenzyl bromide bij de behandeling van 4-nitrotolueen met kalium peroxydisulfaat doet het vermoeden rijzen, dat het door Citterio *et.al.* gebruikte glaswerk niet volledig schoon was.

   Citterio, A.; Santi, R.; Pagani, A., *J.Org.Chem.* (1987), *52*, 4925.

3. Kolasa en Miller hebben ten onrechte verzuimd de optische zuiverheid van de gesynthetiseerde N-hydroxyaminozuur derivaten te bepalen, omdat in tegenstelling tot hun aanname de toegepaste omzetting van een α-aminozuur in een α-acetoxyzuur niet altijd stereospecifiek verloopt.

   Kolasa, T.; Miller, M.J., *J.Org.Chem.* (1987), *52*, 4978.
   Koga, K; Wu, C.C.; Yamada, S., *Tetrahedron Lett.* (1971), 2287.

4. Aangezien in het door Suzukamo en Fukao gepatenteerde proces behalve racemisatie ook epimerisatie optreedt, is het correcter dit proces als een isomerisatie te omschrijven.

   Suzukamo, G.; Fukao, M., *Chem. Abstr.* (1988), *108*, 221334.

5. De publicatie van Brownbrigde en Jowett wekt ten onrechte de indruk dat de toepassing van N-broomsuccinimide in de bereiding van sulfinaat esters uit disulfides nieuw is.

   Brownbridge, P.; Jowett, I.C., *Synthesis* (1988), 252.
   Liskamp, R.M.J.; Zeegers, H.J.M.; Ottenheijm, H.C.J., *J.Org.Chem.* (1981), *46*, 5408.

6. De door Cinquini *et.al.* beschreven bereiding van 2-methyl-1,4-butaandiol uit cis-3,7-dimethyl-1,5-cyclooctaandion is geen ideaal voorbeeld van "la coupe du roi", omdat ten gevolge van de lage regioselectiviteit van de tweede Baeyer-Villiger omlegging de uitgangsstof gedeeltelijk in een bijprodukt omgezet wordt.

   Cinquini, M.; Cozzi, F.; Sannicolò, F.; Sironi, A., *J.Am.Chem.Soc.* (1988), *110*, 4363.

7. Een goed chemicus wast zijn handschoenen.

8. Het ontbreken van een geschikte vrijetijdsbesteding kan bij pas gepromoveerde academici leiden tot een post-doctorale depressie.

   De collega's, Andeno B.V.

Nijmegen, 1 juni 1989 H.J.M. Zeegers